THE HISTOLOGY OF BLOOD AND LYMPHATIC VESSELS DURING THE PASSAGE OF FOREIGN FLUIDS THROUGH THEIR WALLS

II. STUDIES ON ABSORPTION FROM SEROUS CAVITIES

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It is at once evident to any one who studies the enormous literature which has been published during the last century, on the absorption into the body of foreign material from the serous cavities, that the problems presented to the present day investigators in this field may be roughly grouped under four heads, as follows: 1) the localization of absorbing surfaces; 2), the definite establishment of the channels of removal; 3) the determination of the forces concerned in the passage of matter through the walls of the serous cavities, and its entrance into and exit from the vessels which distribute it about the body for storage and digestion, or for destruction and excretion; and 4) the location and identification of the organs concerned in the storage or destruction of substances or fluids absorbed.

Up to the present time not even the questions which are included under the first and fourth of these heads can be said to have been sufficiently answered; the second has been settled definitely only for a single area and with a limited number of substances, and about the third practically nothing is known.

Although a vast amount of work has been done on the localization of absorbing surfaces and their related organs of storage and excretion, until quite recently but little was known regarding them; and this is so in spite of their immense clinical importance in connection with the postural treatment of the various serousitides.

It is owing to the work of MacCallum (1), who made a careful and productive study of the removal of foreign granules from

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the peritoneal cavity through the lymphatic lacunae of the diaphragm, that practically all who have made a study of serous absorption accept it as a fact that a great deal of any solution or foreign body introduced into the peritoneal cavity passes through the peritoneal surface of the diaphragm, and enters the system of the experimental animal or the patient via its vessels.

The position of other foci of drainage has remained unknown, and the tendency to deny or ignore their existence has been and is very strong.

A few observers have suggested the broad surface of the greater omentum as a possible agent in the removal of foreign fluid from the peritoneum, but their assertions have rested upon probability rather than definite proof.

Rubin (2), who attacked the problem from an experimental basis, showed, however, that less fluid was absorbed from the peritoneal cavities of animals whose omenta he had amputated, than from the peritoneal cavities of normal controls; and Crouse (3) after careful study concluded that the omentum is an important factor in the mechanism of peritoneal drainage, and hypothecates a protective lymphatic drainage to account for the phenomena which he has observed. The authors (4) have been able to show experimentally that beyond doubt the omentum is a very efficient agent in the drainage of the peritoneal cavity. By drawing the omentum out of the animal's body through a midline incision, and keeping it immersed in a fluid medium under physiological conditions, it was possible not only to isolate the organ and to prevent the experimental fluid from reaching other surfaces, but also to eliminate any influence on absorption which might be exerted by the increased abdominal tension which follows the intra-peritoneal injection of large amounts of the fluid. In spite of conditions which might be supposed to make for secretion rather than absorption, we found that a large amount of the fluid in which these omenta were immersed passed into the omental vessels, and reaching the general circulation, was carried at once by the blood stream to the organs of excretion, from which the test fluid could then be recovered.

As to the second question, the establishment of the vascular system concerned in the drainage, for example, of the peritoneum, Meltzer (5), Muscatello (6), and others, held that drainage is accomplished through the lymphatics, while Heidenhain (7), Cohnstein (8), Dandy and Rowntree (9), and others, have shown that much of the fluid absorbed from the peritoneal cavity leaves it through the blood vessels. We have never been able to demonstrate the presence of lymphatics in the omental tissue of the adult cat, and Ranvier (10) claimed that while there are lymphatics in abundance in the omenta of young kittens many of them are obliterated by degenerative changes at the age of three months. If lymphatics exist in the cat's omentum they must necessarily drain in the same direction as those of the gastric system; that is, an omental lymphatic stream, if such a thing exists, must eventually become tributary to the lymph content of the thoracic duct.

In the experiments mentioned above with the omentum of the cat, the influence of lymphatic vessels was entirely eliminated in many of our experiments by the ligation of the duct. Hence we were able to prove not only that the omentum furnishes a surface where absorption takes place, but, by varying the fluid in which the omenta were immersed, we have shown that the removal of molecular solutions and colloidal solutions and of fine particulate matter in true suspensions may be accomplished through the blood vascular system to a large extent; though we do not by any means deny the probability of drainage through the lymphatic channels in the localities where these vessels exist. But when any attempt is made to ascertain, through the medium of existing literature, the forces concerned in the absorption of foreign matter from serous surfaces one enters at once into a region of guess and hazard, where only a few isolated facts exist as a guide to certain knowledge. We have only just ceased to argue for and against the presence of preformed 'stomata' and 'stigmata,' and to indulge in surmises as to their physiological significance. Students of the physiology of absorption are still discussing whether absorbed material passes through or between the lining endothelium of blood and lymphatic vessels and the

mesothelial cells of serous cavities. In our own experiments we found that a great deal of fluid may enter the blood stream when the influence of intra-abdominal pressure is removed; and, since our fluids were isotonic with the blood serum of the experimental animal, osmosis as it is generally understood, could have had only a negligible amount of influence upon the phenomena observed. Indeed if osmotic pressure had any influence at all, it would seem that it would have been exerted against rather than for the passage of the experimental fluid into the blood vessels, since even the small amount of fluid lost from the solution by evaporation from exposure to the air, must have changed an originally isotonic to a slightly hypertonic fluid, to which one might expect water to pass from the serum through the vascular wall. If such a passage occurred it in no way interfered with the imbibition of the experimental solution. We know very little about the part played by fluid pressure, the movement of the blood and lymph in their respective vessels, or the influence on serous absorption of the movement of contractile somatic organs, like the diaphragm, or the contraction of the musculature of the vessels themselves. We cannot say what chemical changes accompany or influence the transport of material from cavity to vessel; or whether the cytoplasm of the cells of the serous cavities, or of the blood and lymphatic vessels play any part in the transmission of matter through the vascular or serous walls. And does a disturbed balance of intra- and extra-cellular equilibrium militate for or against absorption? We do not know.

It will readily be seen that the examination of histological preparations made from the omentum during active drainage, may be of great value in strengthening the positive evidence for absorption through the blood vessels, and in aiding us to understand the mechanism of the removal of foreign matter through the vascular wall.

With this end in view, sections have been made and studied of omenta which, up to the time of fixation, had been exposed to and were absorbing all sorts of material from true solutions to mechanical suspensions. A report of the findings in this material is the purpose of the present paper.

By far the most valuable preparations were yielded by omental tissue which had been absorbing an isotonic solution of potassium ferrocyanide and iron ammonium citrate, and which was fixed immediately upon removal from that fluid in hydrochloric acid formalin with a resulting precipitation of prussian blue—the method used by Weed (11) to study the drainage of the cerebro spinal fluid.

An omentum so treated appears in gross to be stained a uniform pale blue except for the fat, and is patterned by an irregular nerwork of an intense dark blue color. It is only necessary to examine the spread preparations with a binocular microscope to be convinced that the network is made up of the omental blood vessels whose lumina are filled with precipitated prussian blue; the picture is strikingly suggestive of a complete blood vascular injection of the omentum with a somewhat dilute prussian blue gelatine mass, and, in fact, we are dealing with much the same thing, since the coagulation of the colloidal proteids during fixation of the blood serum causes the same comminution of the nascent dye stuff which follows its precipitation in pectizing gelatin. In contrast to the general tissue which fills the meshes of the vascular net and which is very pale blue, or uncolored, a wide deeply stained zone of thickly precipitated dye surrounds each vessel.

The capi laries are all filled with prussian blue, even those supplying the perivascular fat being crowded with the dye and the capillary knots or glomeruli which form the support of many of the taiches laiteuse are completely injected. Here and there a capillary may be seen empty or nearly so, perhaps because contraction of its walls during fixation forced the absorbed dye from its lumen.

Of the larger vessels all have a greater or less amount of dye precipitate within the lumen. All are completely filled, but in some the blue color is perceptibly paler than in others. In general the arteries show much less absorption than the corresponding vein, but the depth of the color may not be the same throughout the length of a given vessel. There are often light and dark blue areas present. These preparations show that ac-

tive absorption is going on through the walls of the arteries as well as through the veins, even arteries with thick muscular walls taking part in the general process as will be shown below.

The larger vessels are paler than the smaller, probably because less fluid is taken in through their walls. In other words as the vascular size increases there is a gradually decreasing concentration of the intra-vascular dye solution, the significance of which will be discussed below.

From the point where the vessels begin to be surrounded by a perivascular sheath of fat the pallor of the precipitated dye in the vascular lumen markedly increases, evidently because the advent of the perivascular fat is accompanied by an increased thickness of the vascular wall and a diminished absorption. That the fat itself can have no effect in the decrease is shown by the intense color of the injection mass in the capillaries supplying the fat; and examination of sections shows that the ferrocyanide solution penetrates easily between the fat cells themselves.

Sections of the same material confirm the evidence of total spread preparations. The capillaries, even those imbedded in and sulpplying the fat, are distended with the blue color, and the veins are full of blue precipitate of varying depth of color. It is however not so easy to see the blue color in the larger arteries in sections.

It is possible even in thin sections to distinguish the deep blue perivascular area described above, and to trace its existence to precipitated dye in the intrafibrillar tissue spaces. Individual fat cells are outlined by dye precipitated from the fluid which has worked its way between the cells that it has never penetrated. Coarse precipitates of dye may be seen along the surface of the omentum and adherent to the surfaces of the elastic fibres. In some places the tissue is diffusely stained, and throughout the omentum, cells, probably of the clasmatocyte type are found, like those described by Weed in the meninges, whose cytoplasm is filled with fine granules of prussian blue, their nuclei however remaining uncolored. This intracellular precipitate is the result of imbibition of fluid by the

connective tisue cells, an adsorption phenomenon of the same nature as the drinking in of solutions of high molecular vital dye stuffs which is responsible for the diffuse cytoplasmic coloration seen early in a course of staining.

The blood vessels contain many leucocytes, mostly of the mononuclear type, embedded in the precipitated blue of the injection mass. This prussian blue precipitate in the blood vessels is not the coarse amorphous mass in which that color is usually seen under the microscope, but because the dye was thrown down in the presence of the colloidal serum proteids it is so finely divided as to appear homogeneous except when examined with the highest power immersion lenses with which its finely granular nature can be ascertained. It is in the same physical condition of finely divided suspension as the silver in silver gelatine mixtures of photographers, or the dye granules in injection masses made by precipitating colors in the presence of solidifying gelatin. The intracellular precipitates and those adherent to the surface of the omentum and its component fibres are much more coarsely granular.

The endothelial walls of both capillaries and larger vessels are stained a dark blue. The cytoplasm of the endothelial and mesothelial cells is entirely filled with a fine granular precipitate of the prussian blue, and in some places dye particles are found apparently between the cells, though the walls of the cells are in such close apposition that it is difficult to say with certainty that such is the cause. The cell nuclei are uncolored, and the cytoplasm of the serous mesothelium covering the omental surface is filled with the fine granules of dye which show the track of fluid which has passed through their bodies.

By the time that the dye bearing serum has reached the visceral blood vessels—liver, lung, etc.,—it has become so diluted with blood from non-absorbing parts of the body that it is not possible to follow the course of the chromogen through the body by the examination of sections. Large quantities are present in the kidney tubules, and the presence of the dye in the urine of the animal can easily be demonstrated.

The same conditions prevail, though they are much more difficult to demonstrate, in preparations made from omenta which have been immersed in strong solutions of trypan blue and collargol and colloidal solutions of other metals.

In animals which have been injected intraperitoneally with the chromogen solution—or where certain isolated portions of the peritoneal surface (small intestine or bladder)—have been immersed in, or covered with cyanide-citrate solution, and fixed in hydrochloric acid formalin, the blood vessels and lymphatics directly beneath the peritoneal surface, are found on section to be filled with dye precipitate and to have the same appearance as the omental vessels. Moreover it was possible in the gross to trace the stained lymphatic vessels directly to the lymph nodes into which they drained, and to obtain definite macroscopic evidence of the presence of prussian blue in the lymph gland. This feature of peritoneal absorption will be taken up separately in a later communication.

It is evident then from these histological preparations that there is very active absorption of foreign fluids through the peritoneal blood vessels, not only those in the omentum, but also through those beneath the peritoneum over the gut and bladder. In all probability fluids may be removed from the peritoneal cavity through any area in which blood or lymphatic vessels lie just beneath the peritoneal surface. Furthermore absorption of fluid obtains not only through capillaries, but through vessels of quite large caliber, and through arteries as well as veins, though not to as great an extent probably, because of the greater obstacles to fluid passage offered by the tissues which go to make up the thicker, denser arterial wall.

This is probably the reason for the pallor of the dye mass within the larger vessels, since it would seem reasonable to suppose that their thicker walls would hinder the passage of fluid and make it slower and of less amount. That fluids do pass through is shown by the fact that the wall and its lining endothelium contain granules of stain precipitated from the fluid during its passage into the vessels. That surrounding tissues have no significance in preventing fluid from coming in

contact with these large vessels is shown, as we have pointed out above, by the ease with which it penetrated between the cells in the perivascular fat and filled the capillaries by which the fat is supplied. There is of course the possibility of dilution of the chromogen fluid in the larger vessels as a result of their receiving blood from vessels through which absorption was not going on, but this is unlikely, since the vessels themselves and their entire tributary area were immersed in the test fluid.

The significance of the dark stained areas about the blood vessels is not quite clear. Apparently the solutions are drawn forcibly from the general tissue towards the blood vessels faster than they can be forced through the blood vessel wall, and then, removal being delayed (perhaps by the condensation of the connective tissue in the vascular margin) they are concentrated there. What the forces are which are exerted on the fluid, and what part is played by the movements of the omentum as a whole, the contraction of the blood vessels and the movement of the blood within them, it is impossible yet to say.

The material demonstrates also that while some fluid may pass between the lining cells of vessels on its way to their lumen, by far the larger part goes through the cytoplasm of the cells themselves. The sections are also of interest in that they show how little, if at all, the omentum was damaged during the operative procedure which preceded its immersion in the test fluid. Nowhere is there any sign of exudation or haemorrhage; there is no cellular death, as may be seen by the uncolored nuclei of the various cells; and, moreover, the mesothelial cells of the serous surfaces show no sign of disturbance or desquamation.

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A CASE OF A LEFT SUPERIOR VENA CAVA WITHOUT A CORRESPONDING VESSEL ON THE RIGHT SIDE

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TWO FIGURES

Many instances are recorded in the literature of the presence in human fetuses and adults of two venae cavae superiores, with or without a transverse inter-jugular anastomosis. The presence, however, of a left vena cava superior persisting without a right (the viscera not being transposed) is comparatively rare. I have studied the original descriptions of all the cases of this nature occurring in the bibliographies by Ancel, P. et Villemin ('08),[1] Boyd ('93), Halbertsma ('62), McCotter ('16), Nützel ('14), and Weigert ('81) with the exception of the case of Mäusert ('99) which was not available, and find that thirteen such cases have been previously recorded.

The subject of the anomaly here recorded is a middle aged, well developed male. The right internal jugular and subclavian veins unite to form a comparatively long innominate vein (referred to in this description as the right innominate) which extends obliquely downward and to the left, ventral to the roots of the arteries arising from the aortic arch and unites with the short left innominate vein to form the left vena cava superior. The left vena cava superior crosses ventral to the arch of the aorta, to the left pulmonary artery, and ventral to the root of the lung as it approaches the dorsal surface of the heart; here

[1] The case of Ancel, P. et Villemin ('08) is usually cited in the literature as being one of simple left vena cava superior, is one of a double vena cava. The case of Cheselden (1713) (the correct reference to which is given in the accompanying bibliography), has been difficult to find on account of the frequence with which an erroneous reference has been given.

it reaches the sulcus coronarius and becomes continuous with a large coronary sinus which opens into the right atrium in the usual situation.

A careful dissection was made for a vein representing the right vena cava superior, but no trace was found excepting the terminal part of the azygos which represents that part of the vena cava superior developed from the anterior cardinal. The highest right superior intercostal vein (draining the first space) is a tributary of the right vertebral. The azygos vein is somewhat smaller than normal, but receives the usual tributaries. It opens by means of the persisting caudal part of the anterior cardinal into the right innominate vein about one inch from its right extremity.

On the left side the highest intercostal is a tributary of the left vertebral vein. The uninterrupted hemiazygos system is a large vein (representing the left superior intercostal, hemiazygos and accessory hemiazygos) which opens into the left vena cava superior. Its caliber is nearly as large as that of the normal internal jugular vein. There are two inferior thyreoid veins (one for each lobe), each of which opens into the right innominate vein. The right internal mammary vein empties into the right innominate vein ventral to the termination of the azygos; the left is represented by two veins, the larger empties into the left vena cava superior, the smaller (representing the pericardiophrenic tributary) into the left innominate vein. The cardiac veins are normal in position and termination. The great cardiac is smaller than usual.

The heart is normal in size and position. In the upper dorsal part of the right atrium at the site of the ostium of the vena cava superior, the atrial wall is covered within by musculi pectinati. Below this point the inner surface of the dorsal wall is smooth.

The ostium of the vena cava inferior at the lower and dorsal part of the atrium is normal, there is a faint trace of the inferior caval valve. The fossa ovalis and its limbus are normal, a foramen ovale being absent. Between the large coronary ostium and that of the vena cava inferior there is no intervening space, nor is there a coronary valve. Both from the exterior and in-

terior of the atrium, the two veins appear to communicate with the atrium by a common opening. The tricuspid valve is normal in position and arrangement. The remaining chambers of the heart are normal.

Before offering an explanation for this anomaly, the normal method of development of the veins in question may be briefly recalled. It is well known that the early embryological condition is one in which the veins are symmetrical on the two sides. On either side the anterior cardinal vein unites with the posterior cardinal to form the common cardinal vein (duct of Cuvier), and each common cardinal opens into the lateral part of the sinus venosus (sinus horn) of its own side. The sinus venosus, which is a transversely widened chamber, at first communicates with the common atrium by a large foramen, but during the formation of the interatrial septum, the sinus venosus comes to open into the right portion of the dividing atrium.

Somewhat later in development the terminal part of the subclavian veins (which at first open into the posterior cardinals) migrate cephalad to become tributary to the anterior cardinals. The large trunk on either side caudad of the confluence of the subclavian and anterior cardinal veins becomes the primitive vena cava superior. Each primitive superior cava consists of two regions, a cephalic part originally derived from the anterior cardinal and a caudal part derived from the common cardinal. The smaller trunks lying cephalad of the anterior cardinal-subclavian junction of either side represent the internal jugular of the adult.

Subsequently there is formed upon the medial side of each internal jugular vein, in close proximity to its junction with the subclavian, a vein (Vena thymico-thyreoidea, Thyng '14) which drains a venous plexus about the developing thyreoid and thymic glands. An anastomosis between these thymico-thyreoid veins evidently forms a transverse anastomosis, connecting the right and left jugulars, which normally becomes the vena anonyma sinistra (Szawlowsky '91, Anikew '09, and Thyng '14). This interpretation is substantiated by the fact that the inferior thyreoid and thymic veins of the adult are usually tributary to the vena anonyma sinistra.

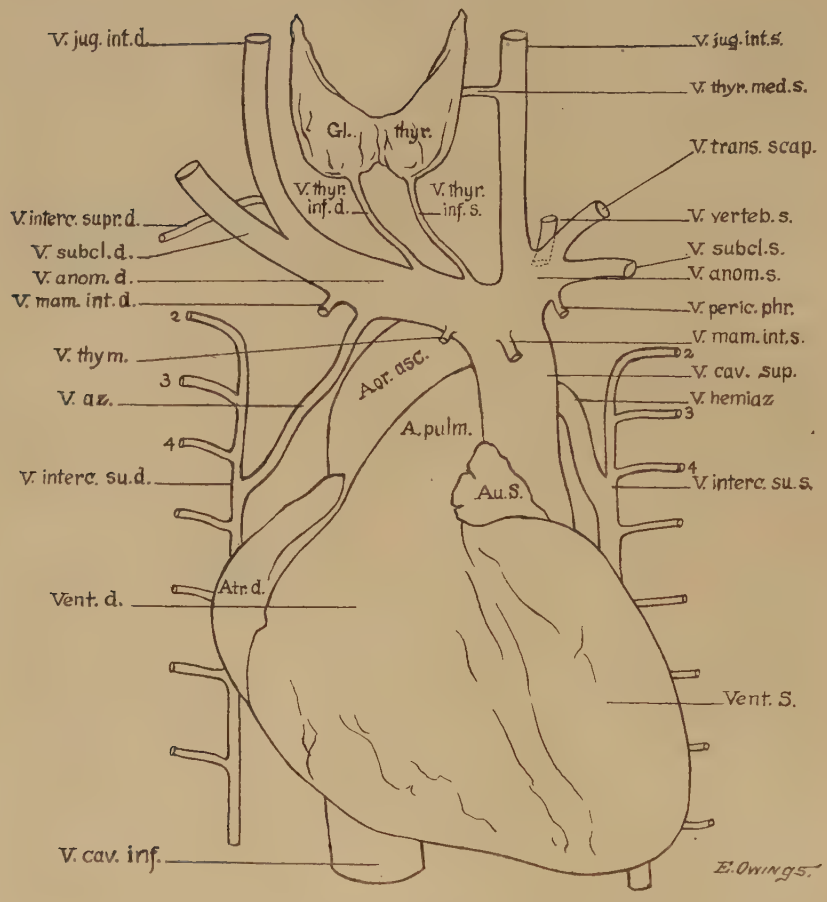

Fig. 1 Ventral aspect of heart and thoracic veins

ABBREVIATIONS USED IN FIGURES 1 AND 2

A.pulm., A. pulmonalis
Aor.asc., Aorta ascendens
Atr.d., Atrium dextrum
Au.s., Auricula sinistra
Gl.thyr., Glandula thyreoidea
V.anom.d., V. anonyma dextra
V.anom.s., V. anonyma sinistra
V.az., V. azygos
V.cav.inf., V. cava inferior
V.cav.sup., V. cava superior
V.hemiaz., V. hemiazygos
V.interc.supr.d., V. intercostalis suprema dextra
V.interc.su.d., V. intercostalis superior dextra
V.interc.su.s., V. intercostalis superior sinistra
V.jug.int.d., V. jugularis interna dextra
V.jug.int.s., V. jugularis interna sinistra

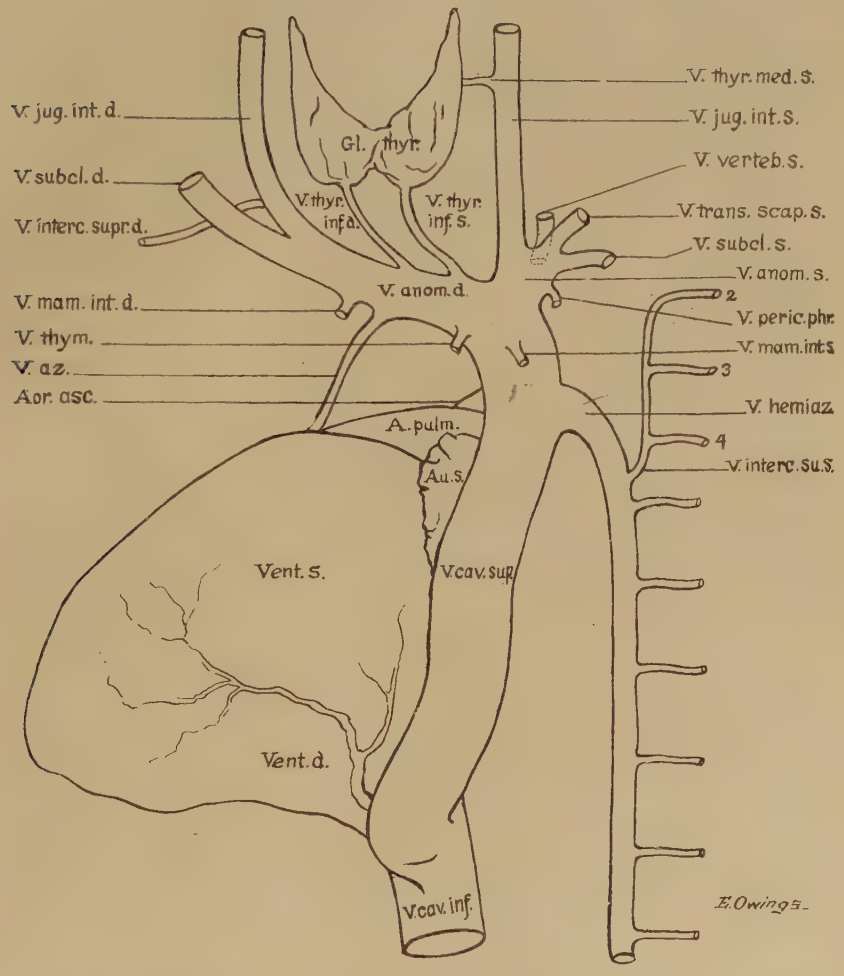

Fig. 2 Ventral aspect of thoracic veins. The apex of the heart has been turned toward the right to expose the left vena cava superior.

ABBREVIATIONS (CONTINUED)

V.mam.int.d., V. mammaria interna dextra
V.mam.int.s., V. mammaria interna sinistra
V.peric.phr., V. pericardiaco phrenica
V.subcl.d., V. subclavia dextra
V.subcl.s., V. subclavia sinistra
V.thym., V. thymica
V.thyr.inf.d., V. thyreoidea inferior dextra
V.thyr.inf.s., V. thyreoidea inferior sinistra
V.thyr.med.s., V. thyreoidea media sinistra
V.trans.scap.s., V. transversa scapulae *sinistra*
V.verteb.s., V. vertebralis sinistra
Vent.d., Ventriculus dexter
Vent.s., Ventriculus sinister

Normally the blood which reaches the left side of the neck now presumably finds a more favorable course through the transverse inter-jugular anastomosis into the primitive right vena cava superior and thence into the right atrium, the greater part of the sinus venosus by this time having been absorbed into the latter. At any rate, the portion of the primitive left vena cava superior, representing the part of the common cardinal immediately caudal of the termination to the hemiazygos system (posterior cardinal), either atrophies or becomes fibrous. The transverse inter-jugular anastomosis then becomes the left innominate vein of the adult; the terminal part of the left anterior cardinal forms the proximal part of the left superior intercostal, and the caudal portion of the left common cardinal persists as the oblique vein which is tributary of the coronary sinus. The adult vena cava superior formed by the confluence of the right left innominate veins, represents the terminal part of the right anterior cardinal together with the entire right common cardinal vein.

EXPLANATION OF ANOMALY

The anomaly above described is apparently due to the fact that subsequent to the formation of the transverse inter-jugular anastomosis, the right common cardinal was obliterated instead of the cephalic portion of the left common cardinal, which here remained intact. This condition may be explained by assuming either that the left thymico-thyreoid vein had a more caudal origin than normally occurs, or that it migrated early in development to a more caudal position than is usual. In either case when the transverse inter-jugular anastomosis was formed, the blood, by following the course of least resistance, must have flowed from right to left instead of vice versa as usually occurs.

I wish to take this opportunity to extend my appreciation to Profs. H. D. Senior and F. W. Thyng of the University and Bellevue Hospital Medical College for their kind suggestions during the preparation of this paper.

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THE INNERVATION OF THE MUSCLE RETRACTOR OCULI

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ONE FIGURE

In view of the great number of dissections of the cranial nerves of the horse that presumably have been made in the veterinary colleges of this country and of Europe and in view of the probably still greater number of similar dissections of certain of our domestic animals, especially the dog, the cat and the rabbit that have been made in the numerous laboratories of comparative anatomy and physiology, it would seem that nothing further remained to be said concerning the gross anatomy of these nerves.

However, after many dissections of the cranial nerves of the horse and certain other of the domestic animals I am convinced that the descriptions of two of these nerves, viz., the N. oculomotorius and the N. abducens as given in many of the standard veterinary and comparative anatomies, are incorrect.

The error referred to consists in attributing two sources of nerve supply to the M. retractor oculi (retractor bulbi, suspensor oculi, posterior rectus, choanoid) namely, the N. oculomotorius and the N. abducens whereas the muscle is innervated exclusively by branches from the latter nerve.

The most common statement as to the distribution of the Nn. oculomotorius and abducens, in quadripeds, is essentially that given by Chauveau as long ago as 1857. According to this author the N. oculomotorius is distributed to the following eye muscles—the dorsal, medial and ventral recti, the obliquus ventralis (or externus), the levator palpebrae dorsalis and the retractor oculi with the exception of its lateral portion; it also supplies one or more motor roots to the ciliary ganglion. The

N. abducens, according to Chauveau, supplies the M. rectus lateralis and the lateral portion of the retractor oculi. (Foltz states that Chauveau subsequently found that the M. retractor oculi was innervated exclusively by the N. abducens). This distribution of the two cranial nerves under discussion is, in the main, correct; the nerve supply to the M. retractor oculi however, as given by Chauveau and a number of other writers is without doubt incorrect.

A brief review of the innervation of the M. retractor oculi as given by several writers, in mammals and in some other animals will first be noted.

M'Fadyean gives precisely the same distribution as just quoted. Bradley's description is the same as the above with this slight difference, viz., the medial part only of the M. retractor oculi is mentioned as receiving a branch from the N. oculomotorius; the lateral portion of the muscle, according to Bradley, is supplied by a branch of the N. abducens, as described by Chauveau and M'Fadyean. In the latest American edition of Strangeway's Veterinary Anatomy no mention whatever is made of any portion of the M. retractor oculi being supplied by the N. oculomotorius. But taken in connection with what is said of the muscle "that it completely envelopes and forms a sheath round the extra cranial portion of the optic nerve" and also in connection with what is said concerning the distribution of the N. abducens "it is distributed to the lateral rectus and the lateral portion of the retractor oculi" one may fairly infer that the M. retractor oculi with the exception of its lateral portion, is supplied by some other nerve than the abducens, presumably by the N. oculomotorius.

In the first edition of his Veterinary Anatomy Sisson mentions both the oculomotorius and abducens as supplying branches to the M. retractor oculi; one portion of the muscle being supplied by a branch from the dorsal portion of the oculomotorius while the dorsal and lateral parts of the muscle are supplied by the N. abducens. In a subsequent edition, however, this error is corrected the M. retractor oculi being described as innervated by the N. abducens only.

According to Share-Jones the M. retractor oculi receives branches from three different sources—from the oculomotorius, the trochlearis and the abducens.

Martin and Franck state that the M. retractor oculi receives its nerve supply from both the dorsal and ventral branches of the oculo-motorius and from the abducens.

In contrast with this statement of Martin and Franck is that of Ellenberger and Baum, also Gurlt, who mention the dorsal branch only of the oculomotorius and the abducens as supplying branches to the M. retractor oculi.

Struska agrees in all respects with Ellenberger, Baum and Gurlt. Leisering says that the lateral portion of the retractor oculi is supplied by the abducens and the remaining portions of the muscle by branches from the oculomotorius.

According to Zimmerl the M. retractor oculi is supplied from the dorsal branch of the oculomotorius and from the abducens but he does not mention the particular portions of the muscle supplied from each of these two sources.

Varaldi gives practically the same distribution as Zimmerl only he does not state from which branch, dorsal or ventral, of the oculomotorius the filaments to the retractor oculi are given off.

In an article on the development of the eye muscles of the pig, Reuter describes the M. retractor oculi as receiving its nerve supply from both the oculomotorius and the abducens.

In the dog Bradley, Ellenberger and Baum describe both the dorsal and ventral portions of the N. oculomotorius as supplying branches to the M. retractor oculi; they make no mention of any branch from the N. abducens to this muscle.

In the second edition of Anatomie des Kaninchen, Krause describes and figures the ventral ramus of the N. oculomotorius as giving off a branch to the M. retractor oculi. Concerning the N. abducens he says that it gives branches to the M. rectus oculi posticus (rectus lateralis). In many mammals, he says the M. retractor oculi is supplied, as in the rabbit, by the N. oculomotorius; in the cat and the calf, however, the N. abducens is the source of the nerve fibers for the same.

Bensley gives precisely the same distribution of these two nerves in the rabbit as does Krause.

Reighard and Jennings say that in the cat the M. retractor oculi receives its nerve supply from the N. oculomotorius and that the N. abducens is distributed to the M. rectus lateralis; there is no intimation whatever that the latter nerve gives any branches to the M. retractor oculi.

Mivart, Wilder and Gage on the other hand describe the M. retractor oculi of the cat as supplied wholly by branches from the N. abducens.

Concerning the innervation of this muscle, Brinton says "the muscle which sweeps the broad nictitating membrane across the bird's eye and the funnel shaped or choanoid muscle (retractor oculi) which surrounds the optic nerve and eyeball of many mammalia are both supplied from this nerve (N. abducens).

Wiedersheim also describes the N. abducens as supplying the lateral rectus, the retractor oculi and the muscular apparatus of the membrana nictitans in sauropsida, thus agreeing in all respects with Brinton's account of the nerve.

From experimental evidence on the live horse and rabbit Foltz asserts that the N. oculomotorius supplies nothing to the M. retractor oculi but that the N. abducens alone supplies this muscle and the lateral rectus. In a note he further states "it is stated in the treaties of Veterinary Anatomy that this muscle (retractor oculi) in the domestic animals is animated chiefly by the common oculomotor." Chauveau has found recently and our experience confirms it, that this muscle is animated exclusively by the oculomotor externus (N. abducens).

According to Owen "in lower Quadrumana a few fibers seem to be detached from the inner part of the origin of the recti to be inserted into the sclerotic nearer the entry of the optic nerve. This is the remnant of a stronger muscle which in other mammals, with few exceptions, surrounds the optic nerve, expanding funnelwise, as it approaches the back of the eyeball; it is called the choanoid, muscle, or suspensor oculi, and is supplied by a branch of the sixth cerebral nerve."

Montane and Bourdelle describe and figure the N. oculomotorius as supplying all the muscles of the eye except the external rectus, the posterior rectus (retractor oculi) and the great oblique. The N. abducens they state supplies the external rectus and the posterior rectus.

Confirmatory evidence of the error of those who described the N. oculomotorius as supplying branches to one or more portions of the M. retractor oculi are found in the distribution of these two nerves in some of the reptiles. In a paper on the development of the musculature of the head and extremities of reptiles, Corning says that in the lizard (Lacerta vivipera) the abducens muscle mass which gives rise to the Mm. retractor oculi and rectus lateralis is supplied by the N. abducens. Precisely the same distribution of these two nerves is given by Hoffman for another lizard (Lacerta agilis) and for the turtle. In the Crocodile also, according to Fischer, the N. abducens is distributed as in the lizards.

In an investigation on the development of the prootic head somites and eye muscles in Chelydra serpentina, Johnson found that the N. abducens supplies the Mm. rectus lateralis and retractor oculi. No portion of the latter muscle is innervated by the N. oculomotorius.

The writer's conviction of the incorrectness of the descriptions of the distribution of these two nerves, as given by several authors, is based on repeated dissections of the nerves in the horse, ox, sheep, pig, dog, cat, and rabbit; in the woodchuck and the badger the nerves were dissected but once. In most cases the nerves were traced their entire length, i.e., from their superficial origin from the brain to their respective muscles. All of the dissections were made with the greatest possible care under a binocular dissection microscope. In the pig the nerves were also traced microscopically in a 42 mm. embryo cut into sections of 20 microns. In all of these animals the dorsal branch of the N. oculomotorius was distributed to the Mm. rectus dorsalis and the levator palpebrae; the ventral branch was distributed to the Mm. rectus ventralis, rectus medialis and to the obliquus ventralis. In all cases one or more small branches were given off

Fig. 1

1. Cut surface of supraorbital process.
2. Frontal sinus.
3. Cut surface of the zygomatic process of the temporal.
4. Cut surface of the malar.
5. Palpebrae.
6. Gl. lacrimalis (somewhat reflected).
7. M. rectus dorsalis or superior.
8. M. levator palpebrae dorsalis or superior.
9. M. rectus lateralis.
10. M. rectus ventralis or inferior.
11. M. rectus medialis.
12. M. obliquus dorsalis or superior.
13. M. obliquus ventralis or inferior.
14. M. retractor oculi or bulbi. (The greater part of the muscle has been removed; only the two extremities, 14 and 14′ are shown).
15. Cut edge of sphenoid.
16. A. maxillaris interna.
17. A. ophthalmica.
18. A. temporalis profunda anterior.
19. A. supraorbitalis or frontalis.
20. Small artery to the mass of adipose tissue in the temporal ossa.
21. A. infraorbitalis.
22. A. orbitalis or malaris.
23. A. buccinatoria.
24. A. sphenopalatina.
25. N. maxillaris, cut and one end turned aside.
26. N. lacrimalis, cut and turned aside.
27. N. supraorbitalis or frontalis.
28. N. nasociliaris or palpebronasal.
29. N. ethmoidalis.
30. N. infratrochlearis.
31. N. trochlearis.
32. Sensory root of Ganglion ciliare.
33 and *34.* N. oculomotorius, dorsal and ventral branches.
35. Small branch from the N. oculomotorius to the M. levator palpebrae dorsalis.
36. N. abducens.
37. N orbitalis or zygomaticus (the peripheral portion has been removed).
38. Ganglion ciliaris.
39. Nn. ciliares.
40. N. opticus
41. N. sphenopalatinus.
42. Ganglion sphenopalitinum showing many small nerves leaving it.
43. N. palatinus anterior or major.
44. N. palatinus posterior or minor.
45. Cut edge of the periorbita or ocular sheath.

to the ciliary ganglion. In no case were there found the slightest indications of filaments from either the dorsal or ventral branches of the N. oculomotorius to the M. retractor oculi as stated by so many.

In all cases the N. abducens, figure 1, 36, supplied all portions of the M. retractor oculi.

Most of the statements regarding the form of the M. retractor oculi and its relation to the optic nerve are somewhat misleading. In some of the domestic animals as the horse, ox, sheep and pig this muscle is not readily divisible into four distinct portions—dorsal, ventral, medial and lateral as it is in the dog, cat and rabbit, but forms a continuous sheet which surrounds the posterior part of the eyeball and a part of the extra cranial portion of the optic nerve. The medial side of the optic nerve for a distance of one and one half centimeters from the apex of the orbit, in the horse, is entirely uncovered by this muscle all the fibers of which are attached to the lateral side of the optic nerve as shown in the figure (*14'*).

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THE ANATOMY WITH ESPECIAL CONSIDERATION OF THE EMBRYOLOGICAL SIGNIFICANCE OF THE STRUCTURES OF A FULL-TERM FETUS AMORPHUS

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NINETEEN FIGURES

INTRODCUTION

Although teratological literature is replete with descriptions of the various types of the fetus amorphus the specimen here studied is of sufficient interest to record, for no description, to which the author had access, was found that entirely coincides with this case. The outstanding characteristics of this interesting group of monsters are according to Ahlfeld ('04) the absence of a heart, normal bilateral symmetry and generally the brain. If the latter is present to any extent at all it is very rudimentary. The subcutaneous tissue is usually hypertrophied, cystic and oedematous. The cord possesses but one artery and one vein.

A partial list of the authorities consulted is the following: Ahlfeld, Ballantyne, Beardsley, Bevill, Brodie, Charlton, Claudius, Embleton, Hall, Herholdt, Houston, Hicks and Baukart, Hirst and Piersol, Jacobi, J. Jackson, J. S. B. Jackson, Le Cat, Lusk, Mall, Marchand, Meckel, Rauber, Schatz, Schwalbe, Simpson, Tiedmann, Von Winckel, Vrolik, Willey and Windle.

The specimen that is the subject of this paper was received by the author, upon taking charge of the department in 1914, from Dr. J. S. Foote, professor of histo-pathology, Creighton Medical College. Dr. Foote came into possession of the monster in 1901 shortly after it was born. It was well preserved in Kaiserling's fluid. The co-twin was a normal female fetus. The period of gestation lasted the full nine calendar months. The

normal fetus was the first to be expelled at parturition. No other clinical data were recorded; the condition of the fetal membranes and maternal deciduae were not tabulated. Ten years ago Dr. Foote heard that the normal co-twin was a healthy, robust girl of five years of age.

GENERAL APPEARANCES, WEIGHT AND DIMENSIONS

A systematic description of each aspect of the fetus follows in the text. The general appearance of the monster was that of an irregularly rounded, potato-shaped, skin-covered mass with the external indications of the two lower limb buds; a well marked outgrowth for the right upper limb and a slight protuberance over the region of the left upper limb. The ventral surface presented a thoracic and a hypogastric elevation. Upon the cephalic slope of the latter the umbilical cord was located which contained but one artery and one vein. The parietal, occipital and mid-dorsal regions carried hair. There was no external indication of a neck. No skeleton would be suspected by palpation. Upon percussion a tympanic note was elicited in the left lower thoracic region. There proved to be a large cavity, (*11*, fig. 18.) in this area, outside the skeleton, in the subcutaneous tissue. The facial and genital aspects, the limb buds and right glandular area were interesting from the embryological standpoint.

The specimen weighed 1130 grams after excellent preservation in Kaiserling's solution for fifteen years. The greatest length from the tip of the frontal region to the end of the rump was 19 cm. (*4* to *16*, fig. 2). The crown-rump measurement was 17 cm. (*1* to *10*, fig. 3). The greatest width through the upper limb buds was 13.5 cm. (*7*, fig. 4). Through the lower limb buds the width was 6.4 cm. The greatest thickness 8 cm. (*5*, fig. 3) was at the mid-ventral point on the line of greatest width. At the location of the external genitalia, the fetus amorphus was 4.5 cm. in thickness. On the ventral surface there were two elevations with an intervening furrow (*6*, fig. 3). The apex of the caudal elevation (*8*, fig. 3) was just below the umbilicus in the hypogastric region. The thickness of the embryo at this point was 6 cm.

SYSTEMATIC DESCRIPTION OF THE TOPOGRAPHY

Right ventro-lateral aspect (fig. 1)

The cephalic part of the fetus is inclined obliquely dorsad, so as to bring more prominently into view and to show the continuity and relationships of the lower limb buds, *12* and *13*, and external genitalia; *9*, body of the clitoris, *10*, glans clitoridis, *11*, ostium urogenital, *15*, right labia majora, of the ventro-caudal aspect of the fetus, with the rest of the ventral surface

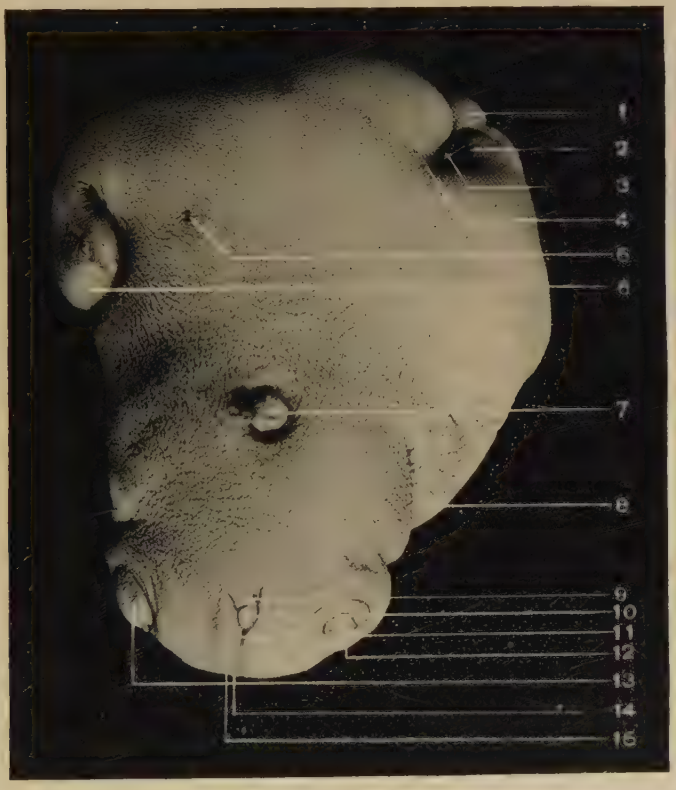

Fig. 1 (About one-half natural size.) *1*, left globular process; *2*, left maxillary process; *3*, oral pit; *4*, groove at ventral junction of mandibular arches; *5*, depression of right glandular area; *6*, right upper extremity; *7*, umbilical cord; *8*, the left ventro-latero-dorsal fissure; *9*, body of the clitoris; *10*, glans clitoridis; *11*, ostium uro-genital; *12*, left lower limb bud; *13*, right lower limb bud; *14*, imperforate anus; *15*, the right labia majora; ventral aspect.

of the body. The embryological consideration of the structures of the external genitalia will be reserved until plate 2 is described. Directly cephalad from the genitals is seen the remnants of the umbilical cord *7*, which contains only one umbilical artery instead of two as are normally found. In the right cephalo-lateral aspect is seen the marked protuberance of the right upper extremity *6*. The slight protuberance, directly across the body on the left side, in the region of the left upper extremity is seen to a better advantage in figure 4, no. *7*. The location of the imperforate anus is marked by *14*.

The interesting fact to be considered in studying this aspect is the depression *5*, of the right glandular area. No similar depression is seen on the left side of the body. This depression is a persistency of the depressed right glandular area, which appears in an embryo of about 25 cm., according to Pinkus ('10) and gradually deepens until it is well marked in a fetus of eight months. At this time the nipple is also supposed to be partly formed, but no sign of a nipple is seen in this specimen.

The genetic significance of the structures of the facial aspect; *1*, left globular process, *2*, left maxillary process, *3*, oral pit, *4*, groove at ventral junction of mandibular arches, will be discussed when plate 1 is considered, figures 6 to 10.

Left ventro-lateral aspect (fig. 2)

This view of the fetus is presented in order to show the continuity of the rudimentary facial structures with the ventral aspect of the thorax and abdomen. The cephalic part of the fetus is inclined obliquely ventrad, in contra-distinction to the position as shown in figure 1, in which the inclination was obliquely dorsad. The most important fact clearly presented is the absence externally of the neck separating head and thorax.

Dorsal aspect (fig. 4)

The left frontal region *1*, is prominently marked. The hair is characteristically arranged over this area in converging whorls. On the other hand the hairs over the crown or vertex *4*, are

arranged in diverging whorls, the direction of the hairs being toward the right, over the left side of crown as can be made out from the photograph. The literature on the subject of the whorls of the hair, together with a complete bibliography, is presented by Pinkus ('10). The external indication of the location of the superior sagittal suture is seen as a sulcus, *3*. The

Fig. 2 (About one-half the natural size.) *1*, right nasal pit; *2*, right globular process; *3*, sulcus between right and left globular processes; *4*, ventral indication of prosencephalon; *5*, left globular process; *6*, left nasal pit; *7*, palpebral folds of left eye; *8*, tongue in oral pit; *9*, left maxillary process; *10*, ventral junction of mandibular processes; *11*, depression for right glandular area; *12*, right upper extremity; *13*, left ventro-latero-dorsal fissure; *14*, umbilicus; *15*, left lower limb bud; *16*, external genitalia, left ventro-lateral aspect.

right frontal and right crown regions are not so clearly differentiated morphologically in the fetus as the left regions. However, the converging and diverging whorls of the frontal and crown areas respectively, are seen, although they do not show so clearly on the right side as upon the left.

The normal and abnormal facial aspects (figs. 6, 7, 8, 9, and 10)

The abnormal facial structures of the monster are exceedingly interesting, due to the fact that they retain the external facial

Fig. 3 (About one-half natural size.) *1*, frontal area; *2*, palpebral folds of left eye; *3*, occipital area; *4*, protuberance over left upper extremity; *5*, cephalothoracic elevation; *6*, ventral depression; *7*, left ventro-latero-dorsal fissure; *8*, ventro caudal elevation; *9*, left lower limb bud; *10*, imperforate anus; left lateral aspect.

characteristics, however considerably distorted, of an embryo of the second month. In the light of normal facial development, as first satisfactorily studied by His ('80), and later by Keibel and Elze ('08), Hertwig ('06), Mall ('91 and '93), Retzius ('04) and others we are enabled to interpret the facts here presented.

Although considerably distorted, the two large globular processes *5* and *10*, developed from the lateral aspects of the median

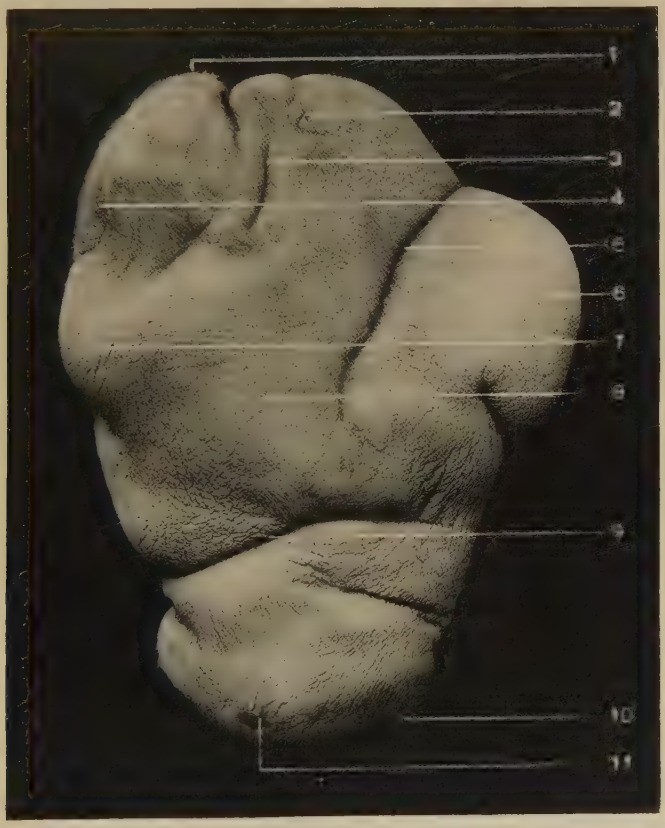

Fig. 4 (About one-half natural size.) *1*, left frontal region; *2*, palpebral folds of right eye; *3*, superior sagittal sulcus; *4*, occipital region; *5*, deep furrow dorso-mesiad of right shoulder; *6*, right upper extremity; *7*, protuberance of left upper extremity; *8*, dorsal line of the body; *9*, ventro-latero-dorsal fissure; *10*, right lower limb bud; *11*, imperforate anus; dorsal aspect.

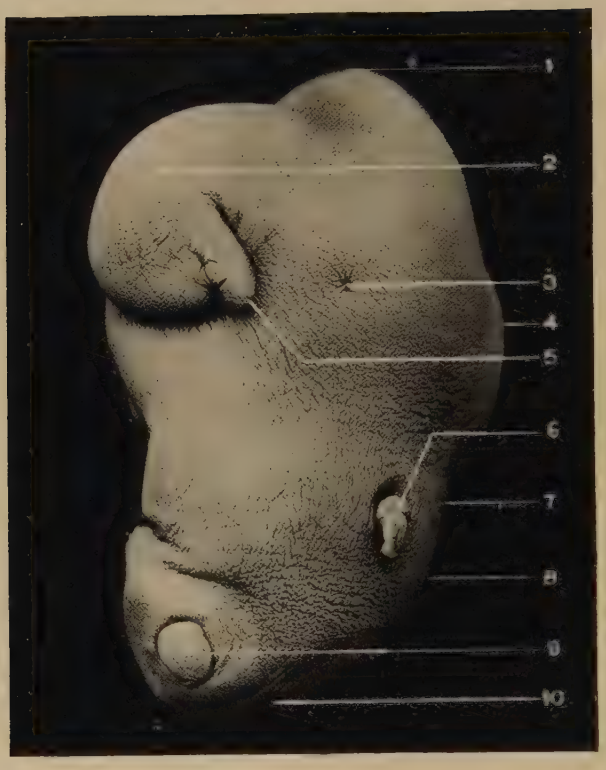

Fig. 5 (About one-half natural size.) *1*, apex of right maxillary process; *2*, right upper extremity; *3*, depression of right glandular area; *4*, ventro-cephalic elevation; *5*, tip of the right upper extremity; *6*, umbilical cord; *7*, ventral depression; *8*, ventro-caudal elevation; *9*, button-shaped right lower limb bud; *10*, location of external genitalia; right lateral aspect.

Figs. 6-10 The indication numbers of figures 6, 7 and 10 mark out similar structures. *1*, crown; *2*, frontal area; *3*, sulcus between the right and left globular processes; *4*, palpebral folds of right eye; *5*, right globular processes; *6*, naso-lacrimal furrow; *7*, nasal pit; *8*, palpebral folds for left eye; *9*, left nasal pit; *10*, left globular processes; *11*, left maxillary processes; *12*, left mandibular processes; *13*, ventral furrow intervening between mandibular arches; *14*, right maxillary process; *7′*, in figures 6 and 7, lateral nasal processes. Figures 6, 7, 8 and 9. Development of the face of the human embryo by His taken from Heisler: figure 6, embryo of about twenty-nine days. The nasal frontal plate differentiating into processes globulares, towards which the maxillary processes of first visceral arch are extending. Figure 7 of about 34 days: the globular, lateral, frontal, and maxillary processes are in apposition; the primitive opening is now better defined. Figure 8 embryo of about the eighth week: immediate boundaries of mouth are more definite and the nasal orifices are partly formed, external ear appearing. Figure 9, embryo at the end of the second month.

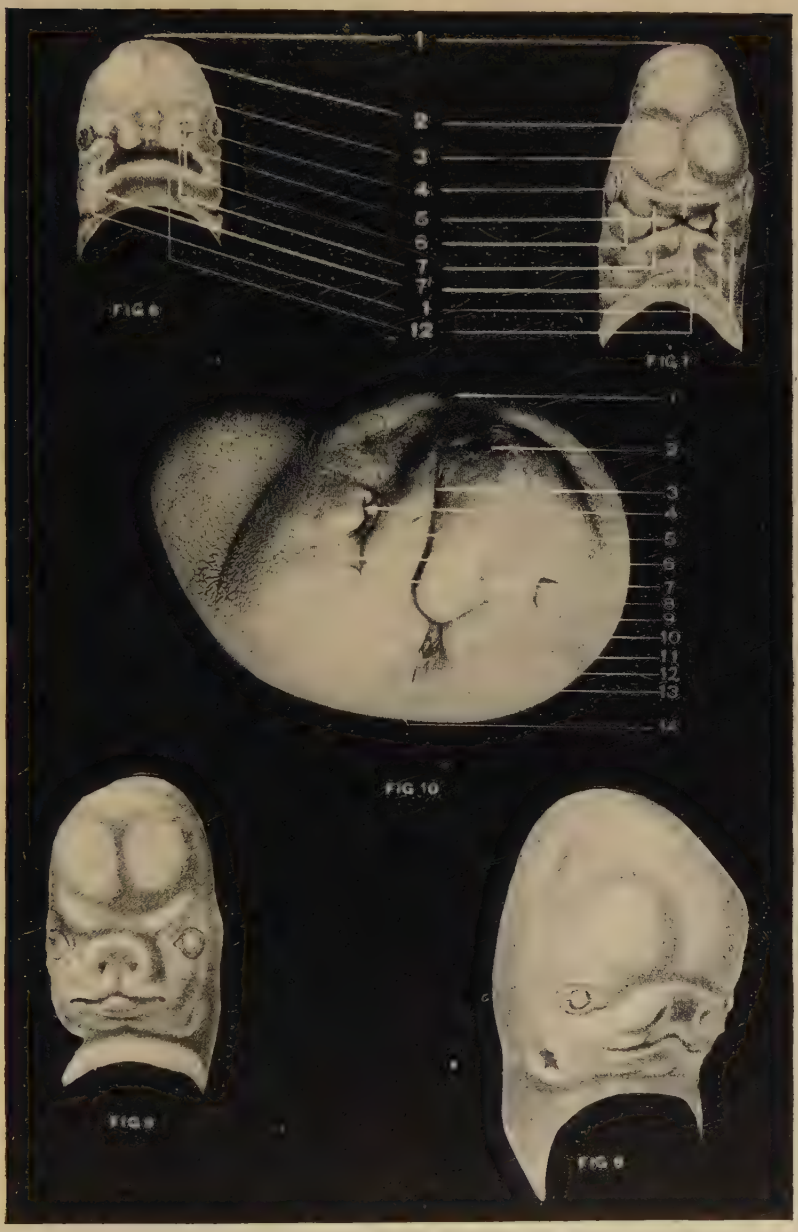
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FIG 10
FIG 11
FIG 12

nasal process are seen separated by a well marked fissure *3*. The right and left nasal pits *7* and *9* respectively are shown as cicatricial depressions. The lateral nasal processes are not distinct on either side. However, their location is differentiated from the maxillary processes by the persistent naso-optic fissures best marked on the right side by *6*. The palpebral folds of the right and left eye, *4* and *8* respectively, are clearly made out. The right is better developed and shows a fissure as does also the left, between upper and lower eyelids.

At the inner angle of the right orbit a groove or furrow continuous with the fissure between the two primitive eyelids is well marked, not so clearly seen on the left side, which courses to the lateral aspect of the anterior nares and is no doubt the persistent naso-optic fissure. The fissure is normally present as seen in figures 6 and 7, No. *6*, but later becomes obliterated as seen in figure 9. On the left side the naso-optic fissure is not so well marked. The right and left maxillary processes, *14* and *11* respectively, are seen as prominent lateral bulgings of the cheeks. The external indication of the left mandibular arch *12*, is apparent, separated by a narrow shallow furrow, *13*, from the external indication of the right mandibular arch. This groove *13*, leads cephalad to the distorted tongue, in the oral pit, shown darkly colored and protuberant in the photograph.

The primitive oral cavity is pentagonal in outline, bounded cephalad by the left enlarged globular process, caudad by the two indistinct external indications of the mandibular arches, and laterad by the external indications of the right and left maxillary processes. The facial aspect thus presents a double hare-lip due to lack of complete fusion of the maxillary and globular processes of their respective sides and the complete split-nose *3*, due to lack of union of the right and left globular processes.

No bony nor cartilaginous structures were found in the mesoderm immediately underlying the superficial facial structures considered in this topographical description. No ears were present.

The normal and abnormal genital aspects (figs. 11, 12, 13, 14 and 15)

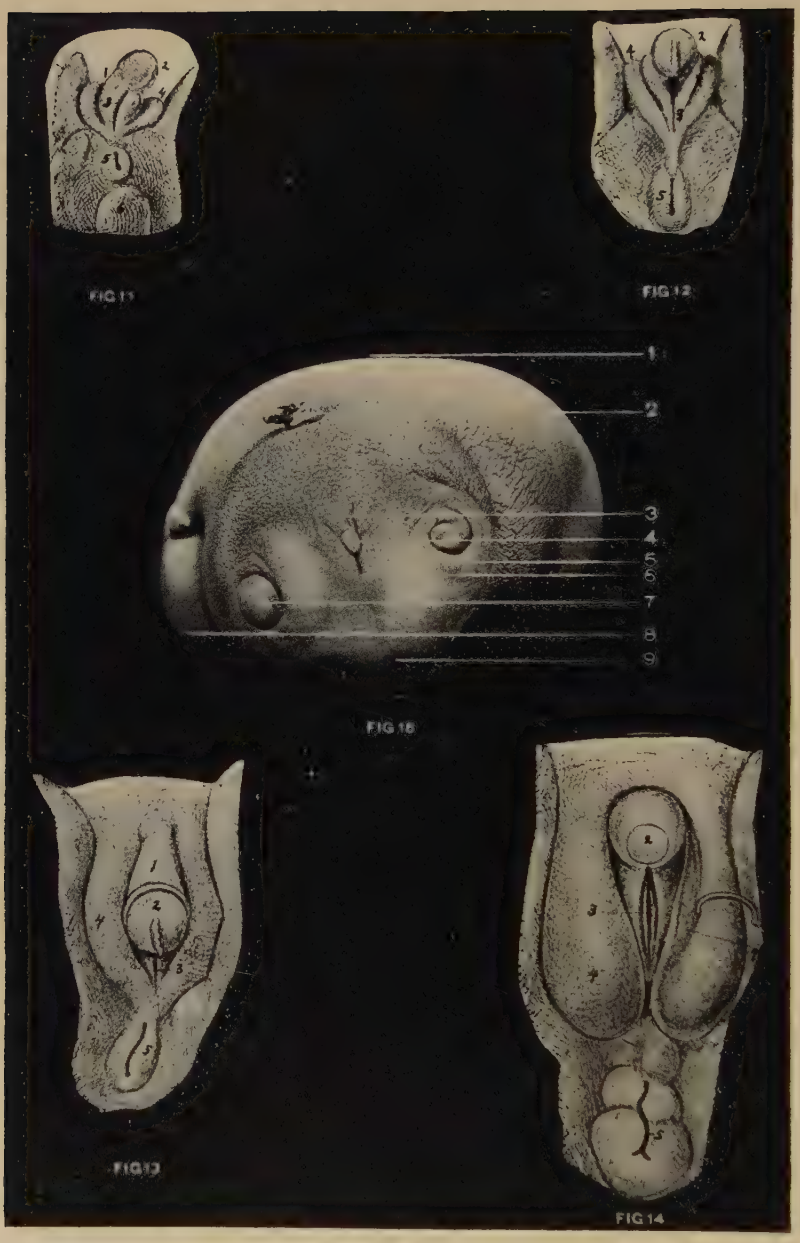
The structures of the genitalia of the monster are interesting, for to a certain degree they retain the characteristics of an embryo, however, considerably enlarged, at the beginning of the third month. At this time the indifferent phallus, by the direction which it takes, shows a transitory sexual differentiation. According to Herzog ('04), the phallus in the female bends downwards and in the male it is perpendicular to the long axis of the body.

By now referring to figure 15, we see an abnormally enlarged phallus *3*, which has practically differentiated into the clitoris. The female phallus at the beginning of the third month may even be larger than that of the male; the downward direction as seen is diagnostic of the female embryo. Surmounting the phallus is the glans *4*, at the base of which is an encircling fold, the praeputium. The urogenital sinus *6*, is seen patent as a groove; the genital folds which bound this groove have receded inwards due to the overgrowth of the genital tubercles or swelling which now form the labia majora, *5*. The urogenital opening distally, towards the glans clitoridis, on the anal slope of the phallus has closed and has formed the urethral groove. Proximally, towards the anus, the urogenital opening *6*, remains patent, which is also diagnostic of the female embryo.

INTERNAL ANATOMY

Subcutaneous and muscular tissues

The fetus was opened by a median incision running to the left of the umbilicus. It was then observed that the skin was firmly adherent to the underlying dense oedematous connective tissue and fat. This tissue was less fibrous as the bony structures were approached. Frozen sections of the tissue contiguous to the skeleton were stained with hematoxylin and eosin. Isolated fibers of voluntary muscle were found intermingled with the connective tissue. There were no well defined groups of



Figs. 11–15 (Figs. 11, 12, 13, and 14. *1*, phallus; *2*, glans clitoridis; *3*, ostium urogenital; *4*, labia majora; *5*, anus; *6*, coccygeal eminence; *7*, labia minora. Fig. 15 *1*, ventral surface of fetus amorphus; *2*, umbilical cord; *3*, body of the phallus; *4*, glans of clitoridis; *5*, left labia majora; *6*, urogenital sinus; *7*, right lower limb bud; *8*, dorsal aspect of right upper limb; *9*, imperforate anus.

musculature. In the irregularly arranged muscular fibers were seen various sized vacuoles, which when stained in an alkaline alcoholic solution of scarlet red fat stain, which is Bell's modification of the Herheimer method, proved to be fat. Counter staining was made with Delafield's hematoxylin and the sections were mounted in glycerin. This method proved an excellent one for the detection of fat within the muscular fibers. The fat vacuoles were found in the angles of Cohnheim's areas and in the spaces between the individual sarcostyles.

Blood vascular system (fig. 16)

The chief features of the blood vascular system of the fetus amorphus are the absence of a heart and, according to Ahlfeld ('80 and '82), the reversal of the flow of blood. In the diagram the arrows indicate the reversed flow of blood according to Ahlfeld's theory. However this idea is refuted by Breus ('82).

The blood enters the body of the fetus amorphus through its single umbilical artery *31*, figure 16. As the latter turns to become continuous with the dorsal aorta, there are two branches given off, *35* and *36*, the left and right common iliacs, respectively. The single umbilical artery is seen to belong to the right side. There is no left umbilical artery present. The right common iliac *36*, branches into the internal and external iliac arteries, *37* and *38* respectively. The internal iliac artery ends as shown diagrammatically in figure 16 in a dilatation representing the capillaries of the pelvic viscera. This vessel evidently carries the blood supply to the viscera as no left internal iliac vessel is present. The right external iliac is also shown diagrammatically as ending in a dilatation representing the capillary system of the right caudal limb bud. The left common iliac *35*, pursues a straight course to the left caudal limb bud where it breaks up into a capillary network.

The next vessel given off as we follow the blood current up through the aorta is the right renal artery *30*, next the left renal *29*, and lastly the hepatic artery *26*, which comes off on the ventral surface of the aorta. At the same level, on the

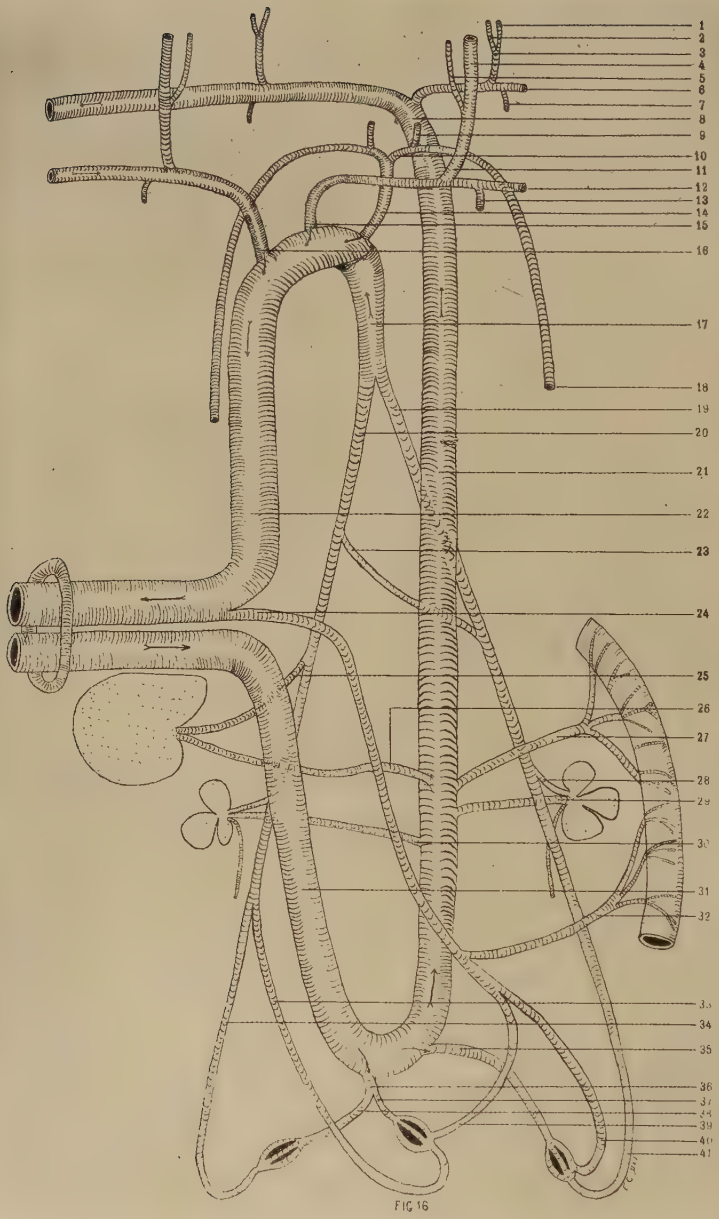

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FIG 16

left lateral aspect, to the origin of the hepatic artery, the superior mesenteric artery *27*, is found. There was no vessel comparable to the inferior mesenteric. The coeliac axis is represented by the single trunk of the hepatic artery. The superior mesenteric artery is a good sized trunk and evidently supplied the regions of the intestine that are normally supplied by the coeliac axis and the inferior mesenteric arteries as well as its own region.

At *8*, the aorta is seen to bifurcate. The right trunk is very much the larger of the two. The branches given off from these two trunks are comparable on each side. The first branch on the left side is the common carotid artery *3*, which bifurcates into the external *1*, and internal *2*, carotid arteries. Caudad the internal mammary artery is given off, *7*, from the subclavian, *6*. From the dorsal aorta the paired intercostal and lumbar arteries which are not represented in the diagrammatic reconstruction, are given off.

The blood is returned from the left and right sides of the head and neck and upper extremities through the left and right superior vena cava *15* and *16*, respectively. The left external *4* and internal *5*, jugulars unite to form a common trunk which in turn with the left subclavian vein *12*, unite to form the left superior vena cava *15*. At the junction of the two veins men-

Fig. 16 *1*, external carotid artery (left); *2*, internal carotid artery (left); *3*, common carotid artery (left); *4*, left external jugular vein; *5*, left internal jugular vein; *6*, left subclavian artery; *7*, left internal mammary artery; *8*, dorsal aorta; *9*, vertebral vein; *10*, point of fusion of left and right azygos veins; *11*, trunk of left common jugular vein; *12*, left subclavian vein; *13*, left internal mammary vein; *14*, common trunk of azygos veins; *15*, left superior vena cava; *16*, right superior vena cava; *17*, common trunk of inferior vena cava; *18*, left azygos vein; *19*, left inferior vena cava; *20*, right inferior vena cava; *21*, thoracic aorta; *22*, umbilical vein; *23*, an anastomotic vein between left and right inferior venae cavae; *24*, diagonal branch of umbilical vein draining the intestinal pelvic, and left lower limb buds; *25*, the hepatic vein; *26*, the hepatic artery; *27*, superior mesenteric artery; *28*, left renal vein; *29*, left renal artery; *30*, right renal artery; *31*, umbilical artery; *32*, superior mesenteric vein; *33*, vein draining pelvic region into right inferior vena cava; *34*, continuation of femoral vein with the right inferior vena cava; *35*, left common iliac artery; *36*, right common iliac artery; *37*, internal iliac artery; *38*, external iliac artery; *39*, vein draining pelvic area into diagonal branch of umbilical vein; *40*, vein draining left lower limb bud into diagonal branch of umbilical vein; *41*, left inferior vena cava.

tioned above to form the left superior vena cava, the left internal mammary vein *13*, joins the left subclavian. The branches forming the trunk of the right superior vena cava are similar to those forming the left superior vena cava but they are not labeled in the diagram.

The left vertebral vein is represented by *9;* the right is not labeled. The regions drained by the left superior intercostal vein and the azygos system of veins are represented by *18*. The right side is not labeled but is similar to the left. The vessels of the two sides after receiving the left and right vertebral veins form a common trunk *14*, which flows into the arch of the umbilical vein. There are no pulmonary veins as well as no pulmonary arteries. The blood is returned from the pelvic viscera through a vessel *33*, which empties into the right inferior vena cava and a vessel *39*, which empties into a diagonal branch *24*, which in turn pours its blood into the umbilical vein shortly before it enters the umbilical ring.

The blood is returned from the left caudal limb bud by two veins, one *40*, which goes to form with the left pelvic visceral vein *39*, the diagonal vein *24*, and one *41*, which is continuous with the left inferior vena cava. From the right caudal limb bud there is but one vein *34*, which joins the vein *33*, draining the pelvic viscera. These two latter veins unite to form the right inferior vena cava and no doubt represent the right and left common iliac veins. Upon the left side the left inferior vena cava does not bifurcate into right and left common iliac veins as is found on the right side. The right inferior vena cava receives the right renal and hepatic veins, *25*. The left inferior vena cava receives the left renal vein *28*. The two inferior venae cavae *19* and *20* unite to form a single trunk which empties into the dorsal aspect of the arch of the umbilical vein. Before these two veins unite there is an anastomotic branch *23*, coursing cephalad from left to right. The blood draining the intestines empties through the superior mesenteric vein *32*, into the diagonal vein *24*, which in turn pours its blood into the common vein of exit, the umbilical vein, *22*.

The abnormal reversal of the direction of the blood stream, through a single umbilical artery, with its subsequent accentuation of certain arteries and degeneration of others, increases the difficulty of interpreting the significance of the vessels dissected. The arch of the aorta is obliterated and at its location we find a bifurcation, with the right integer which supplies mainly the right upper limb, considerably the larger of the two. The left *15*, and right *16*, superior venae cavae are the persistent left and right anterior cardinal veins. Instead of the two posterior cardinal veins joining their respective anterior cardinal veins, they join each other to form a common trunk *14*, which empty into the umbilical venous arch, and is no doubt a persistency of the sinus venosus of the embryonic heart. The inferior vena cava is double *19* and *20*, up to within one inch of its termination, in the umbilical venous arch where its two components of the right and left sides fuse to form a common trunk, *17*. These two posterior venae cavae are undoubtedly the persistent remains of the two subcardinal veins.

There is no portal system comparable to that found in the normal adult. The small rudimentary liver possesses but one artery and one vein. The diagonal vein *24*, draining the blood from the intestines, pelvic viscera and left limb bud is evidently the remains of the portal system. The blood through this vein empties directly into the umbilical vein.

Respiratory system (fig. 17)

The lung tissue was completely degenerated. In the thorax there was found a putty-like mass of degenerated tissue through which the oedematous connective tissue was growing. Broken off portions of the cartilaginous bronchi were found throughout the above mass. The small bronchi which contain portions of cartilage and those in the stage of pre-cartilage, proved to be very resistant. The trachea *3*, is seen tapering to its bifurcation into the right and left bronchi at *5*. There were rudimentary respiratory passages with anterior and posterior nares leading into the dorsal aspect of the deformed mouth. The larynx *1*, is considerably dilated as seen in figure 17.

Digestive system (fig. 17)

The alimentary tract began at the mouth, and at the caudal part of the pharynx dorsad to the larynx the esophagus *2*, figure 17, began. A small dilatation *6*, is detected in the region of the stomach. A duodeno-pyloric flexure is made and at the base of the umbilical ring *7*, and extending into the umbilical cord, a few coils of small intestine 9, were unraveled. The small cecal evagination *8*, was found at the base of the umbilical ring. The large intestine had already made the primary twist across the small intestine. From the cecum the large intestine *13*, is traced to its termination in the rectum *25*. The descending and part of the transverse colons are marked out at this stage of development. The relationships of the intestinal coils to the umbilical vein *10* and umbilical artery *11*, and urachus *12*, at the base of the umbilical ring, are also shown in figure 17. There was no pancreas present. The common bile duct was absent. The rectal cul-de-sac is connected to the utero-vaginal tube by a fissure-like opening *22*.

Uro-genital system (fig. 17)

The urinary apparatus is separated from the genital structures as shown in the figure. Both kidneys *16*, are tri-lobed, the left being slightly larger than the right one. A ureter *17*, leads from each to the bladder, *21*. From the bladder leading to the uro-genital sinus is the urethra *23*. From the tip of the bladder there is a patent tube, the urachus *12*, which leads up to and out through the umbilical cord.

Fig. 17 *1*, The larynx; *2*, the oesophagus; *3*, trachea; *4*, broken off bronchi in thorax; *5*, bifurcation of trachea into the right and left bronchus; *6*, spindle shaped stomach; *7*, umbilical ring; *8*, cecum; *9*, loops of small intestine; *10*, umbilical vein; *11*, umbilical artery; *12*, urachus; *13*, descending colon; *14*, left renal vein; *15*, left renal artery; *16*, tri-lobed metanephros; *17*, left ureter; *18*, fimbriated end of left Fallopian tube; *19*, left Fallopian tube; *20*, dichotomus division of the uterus into the two Fallopian tubes; *21*, bladder; *22*, opening between rectal cul-de-sac and the utero-vaginal tube; *23*, urethra; *24*, vaginal portion of ureto-vaginal tube; *25*, distal end of rectal cul-de-sac.

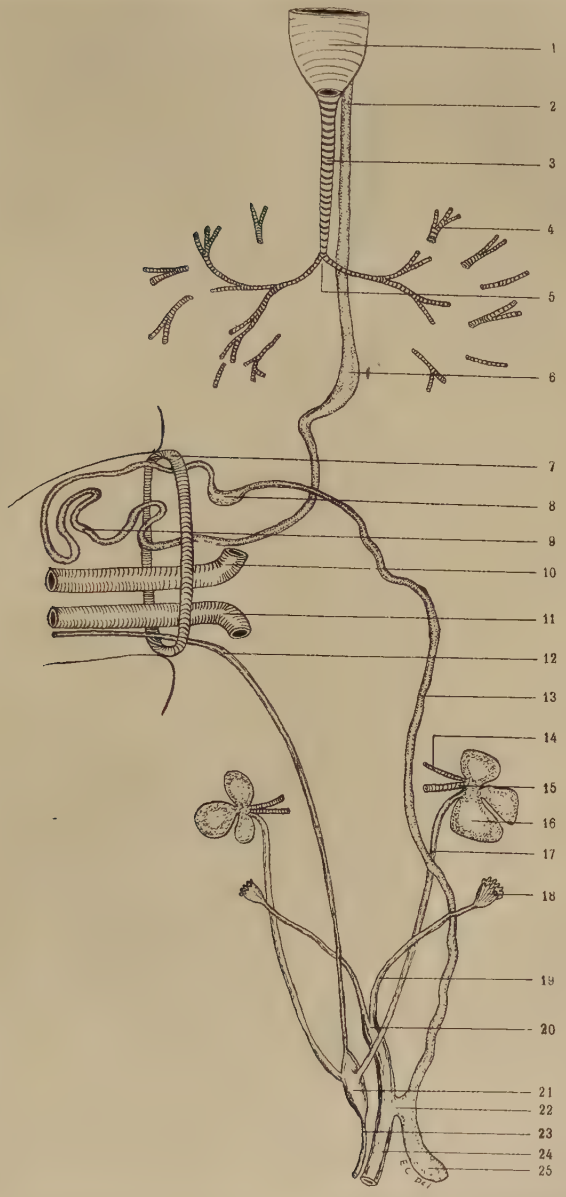

FIG 17

The genital system is composed of a utero-vaginal tube *20* to *24*, continuous with the two oviducts which branch immediately in a Y-shaped manner. The latter end in a fimbriated dilation *18*, the left slightly larger than the right. There were no ovaries. The alimentary tract is connected with the utero-vaginal tube at the location marked *22*, which represents the persistent connection between the alimentary tract and the urogenital systems in the cloaca.

Nervous system

There was an entire absence of the brain and cranial nerves. The upper part of the cord was absent as far as the seventh cervical nerve. From this point to the end of the lumbar vertebra the cord was present. There was a rudimentary cauda aquina extending from the caudal end of the cord. There were irregular net works of nerves in the regions comparable to the brachial and lumbo-sacral plexuses.

Skeletal system

From the tip of the frontal bone, the highest point on the skull, to the tip of the fourth sacral vertebra the bony skeleton measured 14.5 cm. (*2-12*, fig. 19) From the tip of the left shoulder girdle to the tip of the rudimentary right ulna along indication line *9*, figure 18, the width was 8.5 cm. A comparison of the intact fetus amorphus can easily be made with its skeleton when it is remembered that before the dissection the monster measured 19 cm. greatest length and 13.5 cm. the greatest width. At least 2.5 to 3 cm. of dense connective tissue and fat were interposed between the tip of any bony structures and the outer skin which precluded the palpation of the underlying skeleton.

The skeleton was composed of a deformed cranium which was composed of, a fused occipital, sphenoid, ethmoid, temporals, and frontals. The two parietals were respectively distinct Many of the bones of the face and those of the bony vault of the cranium were fused into a distorted mass and could be made

FIG 18

Fig. 18 Skiagraph No. 1. *1*, right parietal bone; *2*, right temporal bone; *3*, occipital bone; *4*, atlas bone; *5*, spine of scapula; *6*, glenoid fossa; *7*, right clavicle; *8*, right humerus; *9*, left scapula; *10*, proximal end of ulna; *11*, left subcutaneous cavity; *12*, first lumbar vertebra; *13*, liver tissue; *14*, first sacral vertebra; *15*, center of ossification of right ilium; *16*, fourth sacral vertebra; *1c*, first cervical vertebra; *1d*, first dorsal vertebra; *1L*, first lumbar vertebra; *1s*, first sacral vertebra.

FIG 19

Fig. 19 Skiagraph No. 2 *1*, fused mandible and temporal bones; *2*, frontal bone; *3*, right parietal bone; *4*, anterior fontanelle; *5*, left parietal bone; *6*, occipital bone; *7*, styloid process of left temporal bone; *8*, left clavicle; *9*, liver tissue; *10*, first lumbar vertebra; *11*, first sacral vertebra; *12*, center of ossification of left ilium.

out only with difficulty and then only by location and vague similarities to the normal bones. The nasal, lacrimal and vomers were completely fused and these in turn were fused to the midpoint of the mandible by the plowshare of the vomer. The mandible was completely continuous by bony union with the temporal bones on each side of the cranium. The paired maxillae, zygomatic and palate bones, six in all, were entirely absent. A rudimentary hyoid bone was present.

The vertebral column presented complete spina bifida from the atlas to the fourth sacral vertebra *16*, figure 18. The laminae were widely separated which deformity is well seen in figure 18 in the dorsal view of the skeleton. In the cervical, most of the dorsal, lumbar and sacral regions the laminae were fused to each other. The atlas was completely fused with the occipital bone at the base of the skull. As can readily be seen by reference to figure 18, the vertebrae presented right lateral scoliosis with the vertex of the curvature at the ninth dorsal vertebra. There is also a slight dorsal kyphosis, sharply angular, from the second to the sixth dorsal vertebrae, somewhat obscured by the opacity in figure 19. The last sacral and coccygeal vertebrae were absent.

Upon the left side of the thorax the first nine ribs were fairly well formed with the remaining three merely attenuated cartilaginous rods. Upon the right side the first seven ribs were fairly well formed and the remaining five represented only by fine rods of cartilage. Upon the ventral aspect the thorax presented a complete cleft sternum. The cartilaginous ribs over the cardiac area presented a distinct bulging.

Upon the left side the upper extremity presented a compound clavicle and scapula the remaining bony structures being absent. Upon the right side in addition to the distinct clavicle and scapula (which are not compound as on the left side) there was a well formed humerus 3 cm. long and the proximal end of the ulna 0.5 cm. long. No radius nor other bones of the upper extremity were present. Both clavicles were completely ossified. The left scapula presented ossification along the acromial process, where it is continuous with the left clavicle. The spine

and supra-spinous fossa were also completely ossified. The corocoid process was not present. The right scapula was completely cartilaginous except the acromial process and contiguous parts of the spine and corocoid process.

The pelvic girdle presented centers of ossification for the ilium, ischium and pubis. The sacro-iliac synchondrosis was cartilaginous on its iliac and sacral aspects. Both pubic elements of the symphysis were cartilaginous. Besides the pelvic girdle there were no other bones of the lower extremity present.

In conclusion, I wish to express my gratitude to Dr. J. S. Foote for the fetus amorphus recorded in this article; to Prof. J. C. Heisler and the Saunders Publishing Company for their permission to reproduce figures 6, 7, 8, 9, 11, 12, 13 and 14 of this article from Heisler's Embryology; and to Dr. A. F. Tyler, professor of Roentgenography, Creighton Medical College, for the skiagraphs herein reproduced.

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THE THORACIC DUCT IN THE RABBIT

RAY HENRY KISTLER

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SIX FIGURES

Notwithstanding the above title I intend to describe not only the thoracic duct, its beginning and termination, but also the large lymphatic vessels in the abdominal cavity which are its real beginning. In my description I will also take into account the main lymph channels from the brim of the pelvis.

The injections were made on a series of rabbits, the exact species of which because of cross breeding I was unable to determine. They were bought at random from rabbit farms where they had been bred promiscuously. I used 26 adult animals about two years of age, sixteen males and ten females. Immediately after killing by illuminating gas the animals were taken from the jar and placed on an animal board. The hind and front foot pads were immediately injected by means of an ordinary hypodermic syringe with a medium-sized needle. I used India ink for the injection mass and found that by inserting the needle just under the skin in the pad of the foot, this fluid would immediately travel on up the leg in the lymphatic vessels. Sometimes the ink would go only as far as the nodes in the popliteal fossa, while in other animals it would go clear up into the abdominal vessels, and even into the thoracic duct. Out of the 26 animals I succeeded in getting the injection fluid to go from the pad of the hind foot clear up the thoracic duct in five cases. In all the rest it stopped at the popliteal nodes. In three of the five cases I found that there was an anastomosis of the vessels around the popliteal node but in the other two cases it looked as though the injection had gone right through the node. The inner part of the node was usually darker than

the periphery and in one instance just one portion of the node was blackened. Whether this can be explained by a difference in the arrangement of the lymphatic vessels or is due to the effect of the strong pressure created by the syringe I cannot say. However, my classmate, Clattenberg, found that young kittens and guinea pigs, in same way, always show the injection clear up the thoracic duct.

It occurred to me that perhaps I did not have enough pressure to force the injection on through. I therefore used a larger syringe, in a few cases, and injected as much as I could into the subcutaneous tissue, but the results were as before for I did not get the injection to go beyond the popliteal nodes in any of these cases.

After injecting the pad of all four feet in this manner I tied the animal to the board and made an incision through the skin from the mandible to the xiphoid. I then dissected out the two external jugular veins, which are very large in the rabbit and ligatured them close to the clavicle, and opened the thorax and tied off the two superior venae cavae. In this way I prevented the injection mass from either going on into the heart or cranially through the large veins. Next I skinned the hind leg and dissected out the popliteal nodes. There may be one or two of these and they are of considerable size. Upon injecting these nodes the ink usually went through into the thoracic duct very easily if the leg was repeatedly flexed and extended.

I also always opened the abdomen and injected the large group of mesenteric nodes in order to distend the duct and vessels so that they stood out distinctly. Injections into the pads of the anterior extremity usually stopped in the axillary nodes. I succeeded in injecting these nodes only a few times and not as easily as the popliteal nodes. In almost every case about 14 or 15 cc. of ink was used per rabbit. Of this 4 cc. was injected from the hind and 3 cc. from the front pads, 4 cc. from the popliteal nodes, 1 to 2 cc. from the axillary nodes and 3 cc. from the mesenteric nodes. Since I was not able to dissect the vessels very well in the fresh specimen the whole animals were preserved in a dilute solution of formalin. After this I could

finish my dissection easily especially that of the duct at its termination into the great veins of the neck.

The injection went through the nodes at the bifurcation of the abdominal aorta to the vessels above. This raises the question as to whether an injection can go through a node. This Baum '11 answered in the affirmative. Baum also always found connections of the lymphatics with the peripheral veins but this was never noticed in my injections.

In my series of animals I found that the thoracic duct and abdominal vessels were very constant and similar in distribution and appearance for only minor variations were observed. Hence a single scheme can represent the arrangement in all the specimens injected with the exception of a few variations which will be spoken of later.

From 3 to 5 lymph nodes were always found at the brim of the pelvis or better at the bifurcation of the large abdominal blood vessels. The more usual number was three nodes. One lay on each common iliac and the third ventral to the bifurcation of the abdominal aorta. However, the position of these nodes varied somewhat for the third or central one was sometimes found in the pelvis just caudal to the bifurcation of the aorta and the iliac nodes also sometimes lay more caudal. The nodes lay in the subperitoneal tissue always ventral to the vessels and could be moved about very easily. They varied in size from 0.5 cm. to 1.2 cm. in diameter and were connected together by vessels thus forming a kind of a plexus.

From this group of nodes a group of 3 to 7 lymph vessels started cranially but in every case small branches of these vessels would connect with the adjoining vessels or a more dorsal vessel so as to form a sort of plexus. Nevertheless I could almost always distinguish and trace a certain number of distinct and parallel vessels which ran cranially as far as the renal vein. This group of lymph vessels was always mainly ventral to but partly surrounded the great abdominal blood vessels. The number and exact position of these lumbar lymph vessels and their relation to the vein and artery I have tabulated in the accompanying table. The average number of lumbar

TABLE 1

NUMBER OF ANIMAL	NUMBER OF NODES AT BIFURCATION OF PELVIS	NUMBER OF LYMPH VESSELS FORMING THE DUCT					NUMBER OF THORACIC DUCTS AT TERMINATION	NODE PRESENT TO RIGHT IN UPPER THORAX	VARIATION AT OR NEAR TERMINATION	PLACE OF TERMINATION		
		In abdominal plexus	Ventral to vein	Ventral to artery	Dorsal to artery	Cranial cisterna				External Jugular	Junction of external and internal	Junction of external jugular and subclavian
1						1	1	+			+	
2	3	4	2	1	1	1	1	−			+	
3	3	4	1	2	1	1	1	−	Dilatation at termination being formed by several other vessels	+	+	
4	3	5	2	2	1	2	1	−				
5	4	4	1	1	2	1	1	−	Loop just before termination.	+		
6	3	7	2	3	2	1	1	+				
7	3	5	1	2	2	1	1	+	Several nodes near termination	+	+	
8	3	4	2	1	1	2	1	−	Divides at 8th rib and joins at 4th forming loop		+	
9	4	4	1	2	1	2	1	+			+	
10	2	3	2		1	1	1	−	Short little vessels going off at 6th and 7th ribs to small nodes to right		+	
11	3	4	1	2	1	1	1	−			+	
12	3	5	2	2	1	1	1	+	Divides above to nodes and two trunks up to the common termination		+	

13	3	6	3	2	1	2	2 from plexus	+ 2 vessels to it	Two vessels from 9th to right then back dorsal to aorta and up through nodes.	1 into innom.vein.		+
14	4	5	2	2	1	1	1	−	Dividing of duct	Int. Jug.		+
15	2	4	2	1	1	1	1	−	Dividing of duct under arch of aorta		+	
16	3	5	1	2	2	2	1	−	Divides under arch and unites at termination		+	
17	2	4	1	2	1	1	2 from plexus	−	Large dilatation 2 ducts from plexus	+	+	
18	3	5	2	2	1	1	1	+	Loop at 3rd rib		+	
19	2	4	2	1	1	1	1	+	Large gland under left superior vena cava 2 cc. long injected by		+	
20	3	5	2	2	1	1	1	−	back pressure. Duct divides just cranial to aortic duct	+	+	
21	3	5	1	2	2	1	1	−			+	
22	3	6	2	3	1	1	1	−	Small vessel off to right at 8th rib to small node. Divides at 3rd one going to left nodes	+	+	
23						2	1	−	Small vessels off to right in thorax	+	+	
24	3	5	1	3	1	2	1	+			+	
25	4	4	2	1	1	1	1	+	Small vessel off to left to node at 12th rib branch at 3rd	+	+	
26	4	6	2	2	2	2	1	−				

lymph vessels was about four. Some might lie ventral and to the right of the vena cava, while others in the same group lay on a level dorsal to the vein but ventral to the artery. Still others lay dorsal to the artery. These vessels could be easily moved and rolled with the blood vessels and their number and position determined although the same vessel changed its relative position in many cases running more dorsal around the vein or artery or more to right or left.

At the level of the left renal vein the group of lymph vessels joined to form two or three vessels which always ran dorsal to the left renal vein and to the left suprarenal gland where they formed the cisterna chyli at the level of the second lumbar vertebra. In this region the mesenteric, diaphragmatic and hepatic lymph trunks join the main lymph vessels. In some cases these join by a common trunk. Generally they flow into a large dilation to the right of the aorta just cranial to the renal veins which is connected to the cisterna chyli by a single trunk or a separate vessel and extend cranially from it and running along the right crus of the diaphragm join the thoracic duct above.

In some cases a trunk from the mesenteric nodes also runs caudally passing ventral to the renal veins and joins the lymph vessels before they reach the cisterna chyli which was almost always distinct. It was a well marked dilatation partly surrounding the aorta, found between the second and third lumbar vertebrae or on the body of the second. It usually lies mainly ventral to the aorta but curves dorsally around the right side. In some cases it was formed by two dilatations one ventral and to the right and the other dorsal and to the left of the aorta which were connected by a large trunk. However, in two cases out of the series of 26 rabbits instead of a distinct cisterna a dense plexus was present. The dilatation into which the vessels from the abdominal viscera terminated was usually located a little cranial and ventral to the cisterna chyli which formed the beginning of the thoracic duct proper. The latter usually left the cisterna by one large trunk which lay dorsal to the aorta and ran in this position through the aortic hiatus in the dia-

phragm up into the thorax always lying to the left and dorsal to the aorta. In some cases a plexus took the place of a single vessel and in others there was a second trunk which started from the ventral part of the cisterna or from the dilatation made by the combination of the visceral lymphatics. This vessel passed to the right side of the aorta along the right crus and at about the 11th intercostal space usually turned dorsally and to the left to join the main trunk. If it did not do this it continued on up on that side of the aorta to a plexus always present at the level of the tenth intercostal space dorsal to the aorta.

The thoracic duct runs up dorsal to the aorta to the level of the 11th rib where it turns to the right and as it crosses always breaks up into a plexus of vessels which lie dorsal to the aorta. The occurrence of this plexus dorsal to the aorta at the level of the 11th rib may be explained by the fact revealed by Lewis ('05) in his work on the lymphatic development in the rabbit. Lewis says that the thoracic duct arises from a plexus of lymphatics surrounding the aorta. Hence it may be that in the rabbit only this part of the plexus persisted into adult life. The duct reappears as a single vessel to the right of the aorta at the level of the tenth rib or a little cranially to it. The dorsal plexus which is made up of from 3 to 5 vessels is always present. As the duct extends cranially on the right side of the aorta and dorsal to it, it lies ventral to the azygos vein. It thus extends to the level of the third rib where it comes to lie on the longus colli muscle. At the level of the second rib it begins to cross to the left and passes dorsal to the aortic arch as one vessel. It then runs up in the interval between the innominate and the left subclavian arteries to its termination into the left external jugular vein at the angle of junction of the internal jugular where the presence of nodes is very common.

Hence forming the main lymph channels of the trunk we find, as shown in figure 1, a group of vessels running up through the abdomen around the large blood vessels and with the visceral vessel forming a cisterna at the level of the second lumbar vertebra. From this on extends one duct which lies to left of aorta to the tenth intercostal space where it crosses forming a plexus dor-

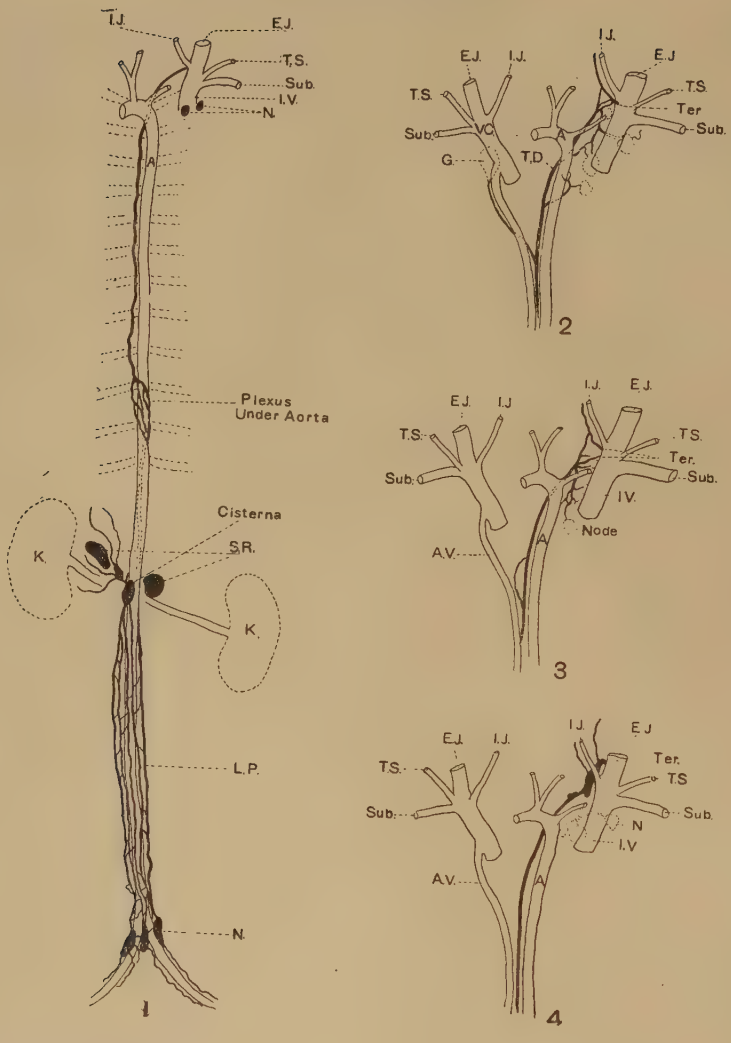


Fig. 1 Thoracic duct.
Figs. 2, 3, and 4 Modes of termination of the thoracic duct.

ABBREVIATIONS

I.J., internal jugular
E.J., external jugular
T.S., transverse scapular vein
Sub., subclavian vein
A, aorta
T.D., thoracic duct
G., gland
A.V., azygos vein
I.V., innominate vein
N, node
S.R., suprarenal gland
L.P., lumbar gland
K, kidney

sal to the aorta. From this plexus a single trunk extends cranially ventral to the azygos vein and to the right of the aorta to the second rib. At this level it crosses to the left again dorsal to the arch of the aorta and extends on up to its termination into the veins to the left as a single vessel.

Variations from the type of duct just described were very few. In 2 out of the 26 cases nos. 13 and 18 I found a plexus in place of a cisterna chyli. The plexus in each case surrounded the aorta and was longer than the average cisterna found in the other cases. In case of one of these the plexus extended on cranially as a narrow plexus on the dorsal side of the aorta up to the plexus at the 10th intercostal space which was described before as being always present.

In 9 cases out of the 26 small vessels were seen in the lower thoracic region running laterally at right angles from the duct to the right and dorsalwards, some lying ventral to the azygos, while others were dorsal. The usual place for these was at the plexus found dorsal to the aorta where the duct crosses over, or slightly above in the eighth or ninth intercostal space. There were never more than four and in most of the cases only one or two vessels. They came off at irregular intervals some close together while others were in the intercostal spaces. In 5 out of these 9 cases they were traced to small nodes found in the intercostal spaces near the sympathetic ganglia. In the other 4 the vessels were small and extended dorsally but it was not possible to determine distinctly where they went and in no case could I trace these to the azygos vein as reported by Witzer ('34) in a human subject and by Boddaert ('99) in the rabbit. Indeed in only one case a vessel was given off from the duct to the left at the level of the twelfth rib. It terminated in a small node. Another variation found was the presence of two ducts in two cases. In each of these instances two ducts came off from the retro-aortic plexus. In one case it came off from the plexus at the tenth intercostal space and ran to the left of the aorta to its termination. This vessel passed through a large group of nodes in relation to the left vena cava, from which it received tributaries and continued on up running dorsal to the

left subclavian artery and also dorsal to the main thoracic duct and terminated cranially to it at the junction of the external and internal jugular veins. The main duct on the other hand terminated at the innominate vein farther down and received a communicating vessel from the accessory duct as it passed ventral to it.

In the other specimen the accessory duct at first turned to the right of the main duct for a length of two centimeters, then curved back to the left and dorsal to the main duct and aorta and ran up on the left side receiving vessels from nodes around the left vena cava and terminating caudal to the main duct in the innominate vein.

Another variation was the presence of a vessel extending from the thoracic duct over to a node of variable size which usually lay under the right innominate vein. This occurred in 10 out of the 26 cases and hence was fairly frequent. The vessel usually came off from the duct at about the level of the third rib and ran cranially and to the right on the longus colli muscle to the node which lay at the first rib. In one case two vessels came from or joined this node one on each side of the right vena cava.

The presence of small communicating loops was noticed in many cases. These were formed by a small vessel branching off from the thoracic duct and then running cranially a little distance and again joining the duct. This occured in about 9 cases at a region between the retro-aortic plexus and the aortic arch. In some instances the loop was longer than in others. In one instance it was about 4 cm. in length but others were shorter even as short as 5 mm. Most of these loops occurred where the duct lay on the longus colli muscle just before passing dorsally to the arch. In some cases small vessels were given off from these loops to surrounding nodes and in one case also ventrally to the aorta.

Figure 2 illustrates the termination of the thoracic duct in rabbit no. 12. It has a single termination at the junction of the left internal with the left external jugular. It divides and is joined by vessels from surrounding nodes which are always present and finally empties by a single trunk into the large vein.

This is the type of termination found in 16 out of the 26 rabbits. In some instances instead of dividing just before reaching the termination the duct formed a large dilation and in others it passed directly through nodes lying in this region. In every case the lymph nodes especially those on the left side and around the three great vessels here illustrated, were completely injected. In some of the cases vessels could be seen coming from the nodes and joining the duct just before it terminated. These were most likely efferent vessels which must have been injected back from the duct. In one case a node fully 2 cm. in length lying dorsal to the left innominate vein was completely filled by the black injection fluid. This indicates that the valves in these vessels were either incompetent or had broken down.

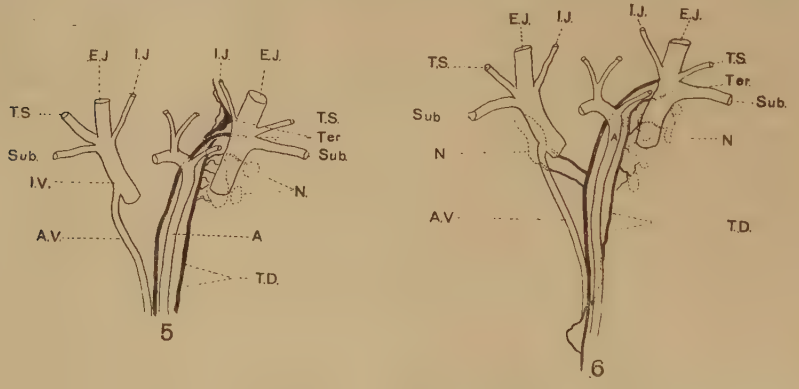
In figure 3 I have illustrated the type of termination found in 5 out of the 26 cases. The termination is double. One trunk terminates at the junction of the internal with the external jugular and the other joins the common jugular vein further caudally. In two of the cases it was hard to determine whether the most cranial duct terminated in the internal jugular itself before the junction was reached or whether it terminated at the junction of the jugulars. In all the five cases it divided after it had emerged from the dorsal side of the aortic arch. Injected nodes were also seen in this region. The termination of the second duct varied a little in its position being close to the main duct in some cases while in others it was a considerable distance caudally.

Figure 4 represents the type of termination found in three out of the 26 rabbits. The termination was single in these cases and was found at the external jugular cranial to its junction with the internal jugular as a rule and usually running dorsal to the internal jugular in its upward course. In two of these cases a large dilation was found just before the duct terminated. From this dilation a lymph vessel always ran up the neck along with the internal jugular vein.

Figure 5 represents the mode of termination of one of the double ducts found in this series. The left duct came off from the plexus dorsal to the aorta at the tenth intercostal space and ran

up on the left side. These ducts empty separately. The one at the junction of the jugulars and the other into the common jugular. After passing through a group of nodes the left duct passes on up dorsal to the subclavian artery and to the main duct to which it sends a communicating vessel and then forms a large dilation which joins the junction of the jugulars. The main duct takes the usual course and empties into the common jugular below.

Figure 6 represents the other case of a double duct found in this series. The accessory duct branched off from the main duct at the eighth rib extending to the right and gradually



Figs. 5 and 6 Modes of termination of the thoracic duct.

curving back to the left, dorsal to the main duct and the aorta. It then passed through a group of nodes to the left and terminated in the innominate vein while the main duct followed the usual course and terminated at the junction of the external jugular with the subclavian vein.

These five types here described include all of my series. McClure and Silvester ('09) have illustrated the termination of the duct in four rabbits and their results are very similar to those obtained by me. In two out of their four cases the termination was single and into the junction of the internal and external jugular veins. This was the predominating place of termination in my series. In their rabbits they found two with double ducts which are similar to those cases in which I found two ducts.

Upon comparing the termination of the thoracic duct in the rabbit with other animals we find a considerable difference. In monkeys as found by McClure and Silvester the most general termination was a single trunk terminating in the jugulo-subclavian junction. According to McClure and Silvester the two jugulars and the subclavian veins in monkeys usually join at a single point. According to Ellenberger and Baum the termination in the dog is usually double. One duct joining the junction of the left internal and external jugulars but the other the external jugular, a condition very similar to the rabbit. The relative size of the position and junctions of the veins is also very much like that found in the rabbit. But according to McClure and Silvester and of Sisson ('11) and of Chauveau ('10) the thoracic duct of the cat usually terminates in the external jugular by two trunks. According to Sisson the thoracic duct in the horse takes quite a tortuous course and usually terminates in one trunk which joins the anterior vena cava just behind the angle of junction of the two left jugular veins. The duct in the horse is said to develop from a plexus and hence varies considerably. Often there are two ducts.

In the ox the duct is said to be very variable, hardly ever being single but often double or plexiform. Its termination also varies being single or double but it usually empties into the junction of the jugular and brachial veins.

Parsons and Sargent ('09) who have investigated about 40 cases in man found that the duct in 75 per cent of the cases terminated as a single trunk. This trunk joins the internal jugular below the left flap of the valve where the jugular joins the left subclavian vein. In only 7.5 per cent of the cases was the termination at the junction. In 22 per cent of the cases the termination was by two trunks into the last centimeter of the left internal jugular vein. In many of the cases the duct bifurcated and then reunited sometimes only in the wall of the vein. In 2 cases out of the 40 multiple terminations were met with but never more than four. Clattenberg found the termination in the guinea pig usually single and into the innominate vein just at the junction of it with the internal jugular.

Nevertheless the duct is usually double up to within a very short distance of its termination. The arrangement of the great vessels is quite different in the guinea pig than in the rabbit.

According to Job ('15) the left duct of the rat empties into the jugulo-subclavian junction but is said to carry lymph from only about half of the body.

Upon comparing my results with those found in other animals I find that there is a considerable difference in some respects while in others the differences are slight. In the horse, it is said, the cisterna chyli is about 10 cm. in length, ampullated and lies dorsal to the aorta and to the right opposite the first and second lumbar vertebrae. It is always very definite and is formed by two trunks from the viscera and one or two lumbar trunks. It seems that the lumbar trunks are not regular vessels but contain nodes of considerable size and are also joined at intervals by lymph vessels. In the rabbit, on the other hand, a group of parallel vessels always run through the abdomen. These are never interrupted by the presence of nodes along their course. They combine and with those from the viscera and the diaphragm form a cisterna chyli which is not always very definite. From the cisterna in the horse the duct runs up through the aortic hiatus and forward on the right of the median plane between the azygos vein and the aorta. It is generally single and extends up to the sixth or seventh thoracic vertebra where it crosses ventral to the oesophagus and then runs to the left of the aorta to its termination.

In case of the rabbit the duct first lies to the left of the aorta and as it passes through the aortic hiatus dorsal to it. It then crosses to the right as described before at the tenth intercostal space and as it does so breaks up into the plexus dorsal to the aorta. This plexus, it seems, is never found in the horse. Cranially from this plexus the duct in the rabbit, lies in the same position as in the horse but crosses to the left, again much higher up and dorsal to the oesophagus.

Comparing the duct in the rabbit with that in the ox we find some interesting variations. Instead of the group of lumbar vessels found in the rabbit there is usually only one large duct

extending up from the lumbar glands and joining the large gastro-intestinal trunk caudal to the renal veins dorsal to which it passes to form a small cisterna. From the cisterna in the ox there may be one or two ducts which may pass through the aortic hiatus or there may be several anastomosing ducts. There hardly ever is only one duct as is typical in the horse. These ducts extend up and the one on the right crosses over at varying heights usually joining the left and terminating as one. In the ox the duct is more variable than in the rabbit.

Comparing the thoracic duct of the human being with that in the rabbit we note few differences. In the abdominal region of man we have two trunks which extend up from the pelvis and lower extremities and the lumbar glands which lie separated by the large abdominal vessels in the midline. These vessels pass dorsal to the renal veins and are joined by a large intestinal trunk which also passes dorsal to these veins and by two descending trunks from the thorax which pass through the ventral part of the aortic hiatus. All of these vessels go to form the cisterna chyli which is about 5 to 6 cm. in length and lies to the right between the aorta and the lower part of the vena azygos, posterior to the right crus of the diaphragm and opposite the first and second lumbar vertebrae. According to Davis ('15) a definite cisterna is only present in 50 per cent of cases in man. From the cisterna the human duct is single and extends cranially through the aortic hiatus. Lying dorsal and to the right of the aorta but ventral and to the left of the vena azygos, the duct terminates singly in 89 per cent of the cases into the left subclavian vein, and in 22 per cent of the cases into the junction of the jugular and subclavian.

In the rabbit, on the other hand, we find a group of 4–6 anastomosing lumbar vessels closely grouped around the large blood vessels with a relatively smaller and indistinct cisterna. From the cisterna in the human being one duct usually extends cranially through the aortic hiatus, lying dorsal and to the right of the aorta but ventral and to the left of the vena azygos. The vessel runs cranially in this position to about the fifth thoracic vertebra. Here it crosses over to the left dorsal to the aorta

and the oesophagus and from there extends out of the thorax on the left side of the aorta.

In the rabbit the duct crosses the vertebral column, once at the tenth intercostal space in the form of a plexus and once above at the second vertebra. This crossing is very similar to that in the man but a plexus is never present in the latter where the duct crosses the vertebral column. Of the many variations which have been found in the human subject none known to me have the characteristics found typical in the rabbit.

Comparing the cisterna of the rabbit with that of the cat we find that in the cat the cisterna is formed by the intestinal trunks and two or three lumbar trunks. It is definite and lies opposite the second lumbar vertebra. From the cisterna the thoracic duct which may be looped extends up as a single duct but always lies to the left and terminates in the left external jugular vein.

In Bos taurus according to Baum, the cisterna is not very large. It is formed by two lumbar trunks which join the visceral trunks caudal to the renal vessels. These vessels join and form one large trunk which runs dorsal to the renal vessels to the cisterna opposite the second lumbar vertebra. From the cisterna the duct runs to the right of the aorta to the fifth thoracic vertebra. Here it crosses dorsal to the aorta and extends up on the left and terminates into the left external jugular vein.

In the dog the cisterna is relatively very much larger than in the rabbit. It is long and ovoid and even extends into the thoracic cavity between the crura of the diaphragm. From the cisterna the duct is usually single, extends up on the right and then crosses over at about the fourth or fifth thoracic vertebra to terminate in the left external jugular vein.

In the guinea pig according to Clattenberg a definite cisterna is not always present. The abdominal vessels are plexiform extending up in the midline. At the level of the renal vessels a number of nodes are usually found in which the plexus is lost. From these nodes extend vessels which form a dilation opposite the first and second lumbar vertebrae dorsal to the blood vessels.

Cranially from the cisterna another plexus of vessels may leave the nodes just mentioned and pass directly into the plexus above the cisterna. This plexus is usually wide and extends up into the thoracic cavity to the level of the eighth rib. From the eighth rib on up Clattenberg almost always found two separate ducts one on each side of the aorta. The one on the right side crosses over at the third rib and forms another small plexus with the left. Cranially from here a single duct which terminates at the junction of the innominate and the internal jugular veins on the left is found. This form of duct is much like the embryonic plexus found in most mammals.

In conclusion I wish to thank Professor Meyer for the help he gave me in the course of this investigation.

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SOME RESULTS AND POSSIBILITIES OF EARLY EMBRYONIC CASTRATION

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SIX FIGURES (FOUR PLATES)

The works of Eigenmann (7, 8), Hoffmann (11), Nussbaum (13), and Beard (3) have supported the proposition that the germ-cells in several vertebrates are precociously segregated, and that their locus of origin is not necessarily the 'germinal epithelium' or even its immediate region. The classic case of early segregation of the germ-tract of a vertebrate is that described by Eigenmann, who was able to trace the lineage of the sex cells of *Micrometrus aggregatus* probably as far back as the fifth cleavage; this is the farthest that any vertebrate germ-cell has ever been traced. Hoffmann discovered the existence of primitive ova in the mesenchyme of a number of bird embryos at a time prior to the establishment of germinal epithelia in those embryos. For a review of the works of Beard (3), Nussbaum (13), Rubaschkin (14), von Berenberg-Gossler, Allen (4, 1, 2) and Fuss (9), and others, the reader is referred to the introductory discussion by Swift (18). For a discussion of the segregation of the germ-cells in the invertebrates, Hegner's 'Germ-Cell Cycle' may be profitably consulted.

The work of Hoffmann and others seems to be borne out by the highly interesting work of Swift (18). The latter has not only strengthened our belief in the extra-regional origin of the sex-cells in the chick, but he has offered a solution of the question why the primordial germ-cells of the chick had never been found prior to the twenty-two somite stage. He maintains that the sex-cells originate in a crescent-shaped area of the extra-embryonic blastoderm anterior to the body-axis at the line of

demarcation between the areas pellucida and opaca (see his figure 15); that these primitive sex cells reach the gonad partly by their own wandering, but principally by way of the blood-stream which transports them either to the gonad where they continue to develop, or to some other region where they soon degenerate. He makes the highly interesting suggestion that the large wandering cells which Dantschakoff (5) found to degenerate in the blood-stream following the twenty-two somite stage are really germ-cells which failed to become incorporated into the gonad.

At a very short time subsequent to the publication of the work of Swift, Professor McClure in 1914 suggested to one of his students that this work of Swift's could be verified or disproved by the early excision of this crescent-shaped area described by that author, provided the operation be done at a time before the germ-cells have wandered away from this area of proliferation. The work was attempted but no results were obtained. Since then the writer has spent a great deal of time trying to develop a technique such that the embryos might be maintained despite the extremely low viability of the experimental material. In this work, some few thousand chick embryos have been sacrificed. During the first year of the work it was possible to rear a few embryos to the seventy-two hour stage; later than this, two embryos survived to the age of five days. These were, however, discarded on the assumption that they were unfit for critical study owing to the fact that they had died a short time before they were preserved. This is explained by the fact that such embryos were always allowed to develop as far as they would. It was not always possible to find them in the act of dying. It was found, however, that in normal individuals which were bled to death and then allowed to stand many hours before preservation that the germ cells were quite easily distinguishable in these corresponding stages; material which has been dead a few hours is of some value, although it is unfavorable for the distinguishing of such fine structures as mitochondria. I have tried to rear the embryos to the more advanced ages for the reason that my chief interest lies not in

the confirmation or disproval of the work of Swift, but rather in determining the later effects of this extraordinary early castration. It occurred to the writer that if this crescent-shaped area is the seat of the keimbahn, its removal would afford a means of the earliest castration ever yet performed on a vertebrate; it would give an opportunity for studying in pure culture the interstitial cells of the gonad—particularly their supposed effect on the secondary sexual characters. If, as castration of animals subsequent to hatching or to birth has shown, the secretion of the gonad profoundly affects the secondary sexual characters, it is reasonable to suppose that it might affect some of those sexual characters which are usually considered as primary —such as the persistence or degeneration of the Wolffian and the Müllerian duct. If the latter be true, early removal of the primordial sex cells might possibly cause the retention of the original ground-plan of the urogenital system, namely the simultaneous existence of both of Wolffian and Müllerian ducts (or at least one of the latter), if however the development and normal functioning of the interstitial cells were in no way impaired by the absence of sex cells, their secretions would cause the alteration of the ground-plan if this were their normal function. But on the other hand, if the gonad were incapable of producing a secretion in the absence of sex-cells, opportunity would be afforded of studying such response or irregularities as might be observed in the development of the other ductless glands.

One castrated embryo was recently reared to an age at which one or the other set of genital ducts should have been eliminated. The ground-plan was found to be persistent when the embryo was dissected, but there is a possibility that an arrest of development due to operation played a part in the result.

An ideal way to confirm the work of Swift would be to transplant a 'crescent' from an embryo of one pure breed to the mesenchyme of an embryo of a different breed but of the same sex. If such an embryo could be reared to the adult stage its germ-cells could be tested by breeding. I have been able to rear an embryo into which a crescent was transplanted, to the age of twenty-one days when it died the day on which it should

normally have hatched. Dissection of this embryo showed it to be a male.

Artificial hermaphroditism would be produced in fifty per cent of such transplantations as this just described.

These are some of the problems raised by Professor McClure's suggestion, and which are now in progress of investigation.

Some results of this work at its present status may be of interest. I shall describe at this time the results of some experiments by means of which I have convinced myself, at least, that the work of Swift has given us a more correct and more ultimate solution of the origin of the germ-cells than any other yet given in case of the chick; that his work is of the very greatest importance owing to the experimental possibilities which have arisen from it. The description consists of a comparison of a few individuals in which castration was complete or at least very nearly so, with normal individuals at corresponding stages.

According to Swift, most of the germ-cells have assembled at the base of the mesentery back of the twenty-second somite when the embryo has reached the stage of thirty-three somites. In this position they remain until the germinal epithelium begins to thicken. They then migrate into the gonad. This is seen to be in process in my figure 1. In most of the adjoining sections, primitive ova are being incorporated into this thickened epithelium. The sections of this embryo are only five micra in thickness, yet it is impossible to find a section in the gonad-region which fails to exhibit sex cells. Some of these cells are found far ventrally in the mesentery. The figure shows the slight protuberance of the right gonad; the region illustrated is very similar in location to that of figure 3 and the small rectangle in the keyplate for the latter. In the left upper corner of figure 1, a few erythrocytes have been inserted; they are drawn to the same scale as the rest of the figure. In every case, erythrocytes were used for comparison with the size of the sex-cells.

Figures 2 and 3 are from a section of the left gonad of an embryo slightly older than that from which the preceding figure was made. The gonads of the two embryos were, however, about equally prominent. The germinal crescent of this em-

bryo was excised at a time previous to the establishment of any intersomitic grooves and at which the neural folds of the brain were first indicated. The embryo was killed at the age of ninety-four hours. In this section it will be seen that the germinal epithelium is very little thickened. As in all other sections of this embryo, germ-cells are entirely absent. Only a few cells in the mesenchyme approach in size the erythrocytes shown in the figure; these are not germ-cells. None can be found which exceeds the size of the erythrocytes to any such extent as do the germ-cells which are invariably present in sections of the gonads and mesentery of embryos of this age, and which can be most readily identified, no matter what may be the technique of fixation or staining employed.

Figure 4 is a section through the left gonad of a normal chick embryo at the age of one hundred and nine hours. This excellent series was prepared about fifteen years ago by Prof. A. M. Miller. The gonad protrudes considerably into the coelom. Among the very compact interstitial cells are numerous lightly staining germ-cells of large size. The mesothelium of the mesentery adjacent to the gonad is very greatly thickened, darkly staining, and as in all normal individuals of this age, contains numerous ova which cause local protrusions into the coelom. It will be noted that these germ-cells which have been incorporated into the mesothelium are slightly larger than those which have reached the gonad. Lying in the mesenchyme of the mesentery are large germ-cells. These can usually be found in any section of a mesentery at this age. Pictures quite similar to this are readily obtained in embryos one hundred and twenty-five hours old except that the sex cells found in the gonad are relatively somewhat smaller. It will generally be found that this size-relationship obtains in normal embryos. The germ-cells which remain in the mesenchyme are usually the largest. It may be that those in the gonad undergo more rapid multiplication. A few erythrocytes sketched indiscriminately from the dorsal aorta are inserted for comparison of size.

Germ-cells can be found in the mesenchyme of the mesentery as late as one hundred and seventy-five hours. This is the oldest material which I have examined for this particular point.

In these older stages, many instances will be found in which nuclear division in such germ-cells has not been accompanied by cytoplasmic division, so that a large multinuclear cell results, having a diameter about equal to that of the mononuclear germ-cells of the thirty-three somite stage. Such a condition is shown in figure 5. A very interesting condition is found here; it will be noted that a large binucleate cell-body projects from the mesenteric surface and touches the gonad from which there is likewise a slight protrusion and on which there is also an interruption of the mesothelium. These are undoubtedly germ-cell nuclei. If this is a case of chemotaxis it is a very remarkable one. It is entirely possible that such proliferations from the coelomic wall might sometimes be misinterpreted as giant blood-cells. In the gonad will be seen a germ-cell which is smaller than those lying in the mesenchyme.

We may now consider the conditions which obtain in a castrated embryo five days old. The operation was performed just prior to the establishment of the first intersomitic groove. If castration was not complete, it was very nearly so. In this embryo I have found about five cells which are considerably larger than the average erythrocyte, and several which are a little larger. I am convinced however, that these are not germ-cells. If one examines Swift's (18) figure 6, for instance, he will find at least two mesenchyme cells in the left upper corner of the figure which are larger than the erythrocyte in the small endothelial cavity at the lower right corner. There would be little danger of confusing these with the large germ-cells present in the figure. I have made no attempt to diagnose germ-cells on the basis of the form of the mitochondria, since there is not perfect agreement on the question whether the germ-cell mitochondria of the chick are characteristic. See Rubaschkin and Tschaschin (14, 15, 16, 17, 19) and Swift (18) p. 496.

The conditions in this five-day castrated embryo are illustrated in figure 6. The section passes through the right gonad. The mesothelium of the mesentery consists of a single layer of cells. In all normal individuals at this age this mesothelium near the gonad is found to be greatly thickened, stains darkly and contains sex-cells. There are no germ-cells in the mesenchyme of

the mesentery, whereas they are invariably present in the normal individual. The gonad contains only interstitial cells, so far as those who have examined the material have been able to detect. The gonad tissue is greatly vacuolated, while in normal embryos even at younger stages the gonad is quite compact and remains so even through rough histological treatment. The interstitial tissue displays solid darkly staining lines of intercellular substance bordered by interstitial cells the arrangement of which gives a foliage-like appearance. In some cases the plane of section passes through an interstitial cell which projects into a large vacuole. When the diameter of such a vacuole is about that of a germ-cell the picture is somewhat similar to that which a germ-cell in a normal gonad would present if the cytoplasm were dissolved out. Professor E. G. Conklin has examined this material and has pronounced the fixation to be sufficiently perfect that germ-cells should be easily distinguished if they were present. If any are present they are certainly very few.

In the foregoing account, the term 'stroma tissue' might well have been used instead of the term 'interstitial tissue.'

It seems reasonable to believe that the abnormal conditions recorded and the early removal of the crescent-shaped piece of blastoderm are causally related. In conclusion I wish to thank Professors C. F. W. McClure and E. G. Conklin for the aid which has made this work possible.

SUMMARY

1. The extra-regional origin of the germ-cells of the chick may be regarded as highly probable.

2. The early location of the germ-tract on the yolk-sac gives opportunity for the earliest castration yet performed on a vertebrate. It makes possible an analysis of the functional activity of the sex-cells and the interstitial cells in the production of internal secretions, the effects of the latter on the sexual characters and on other characters. It makes possible the production of artificial hermaphroditism, provided the germ-cells of one crescent can be made to enter the gonad of an embryo of the opposite sex. The work of Swift is of very great importance.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Section through the right gonad of a normal chick embryo ninety hours old. Picro-acetic fixation. Iron haematoxylin followed by methyl blue-eosin. × 800. In most adjoining sections, the germ cells have entered the germinal epithelium. This section is not typical, but it illustrates an interesting condition.

2 Low-power sketch of the section from which figure 3 is made. The rectangle to the left of the mesentery indicates roughly the regions from which figure 3 is taken. *G. epth.*, germinal epithelium; *Mst.*, mesentery; *Pr.O.*, primitive ova.

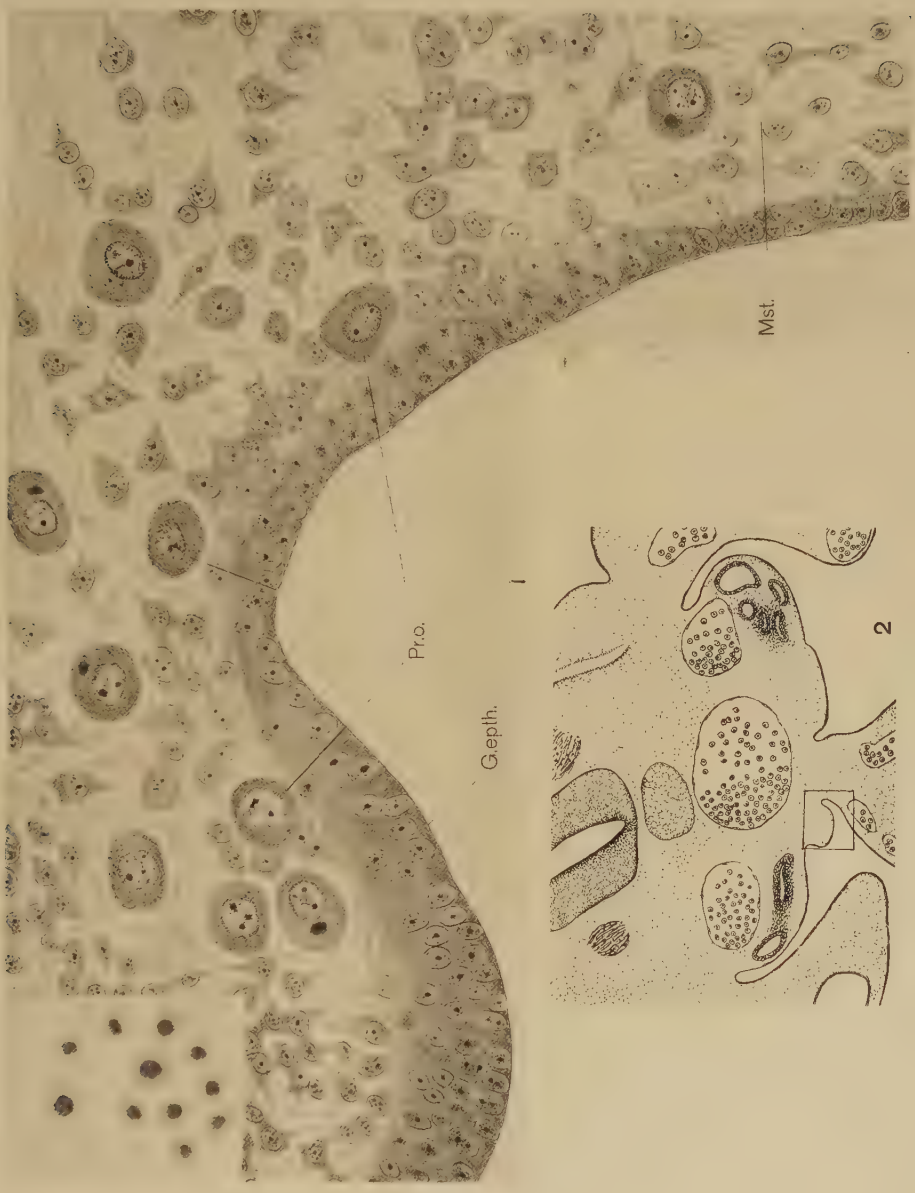
Mst.
Pr.o.
G.epth.
1
2

PLATE 2

EXPLANATION OF FIGURES

3 Section through the left gonad of a ninety-four hour chick embryo which was castrated prior to the formation of any intersomitic grooves. Note the absence of germ-cells and the thinness of the germinal epithelium. The section is viewed from its *posterior* surface. Benda's fixation followed by Meves' iron-haematoxylin stain. × 800. *Endth.*, endothelium; *Erth.*, erythrocytes; *G. epth.*, germinal epithelium. *Msth.*, mesothelium.

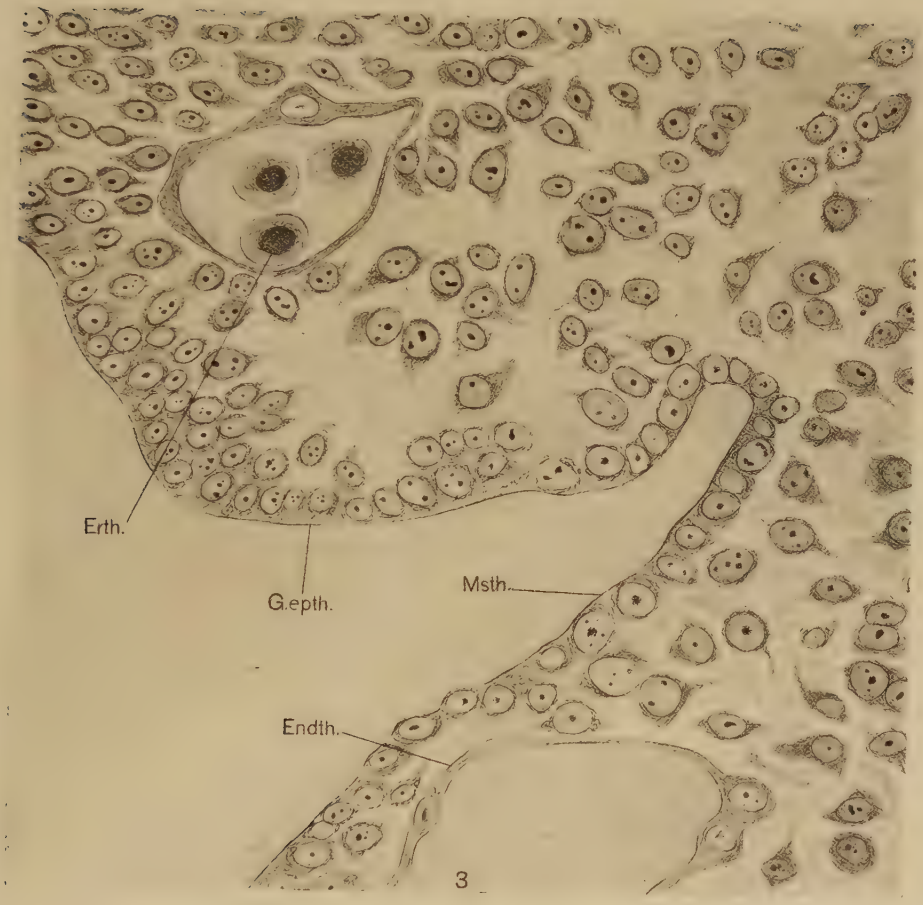
Erth.
G.epth.
Msth.
Endth.
3

PLATE 3

EXPLANATION OF FIGURES

4 Section through the left gonad of a normal chick one hundred and nine hours old, showing germ-cells in the gonad, and in the mesothelium and the mesenchyme of the mesentery. Zenker fixation. Delafield's haematoxylin and orange-G. Princeton Embryological Collection, series no. 46. × 800.

5 Section through the left gonad of a normal chick one hundred and sixty-two hours old, showing primitive ova in the gonad and in the mesentery. Fixation and stain as in figure 4. Princeton Embryological Collection, series no. 41. × 800. *Coel.*, coelom; *Gon.*, gonad; *Mst.*, mesentery; *Msth.*, mesothelium; *Pr.O.*, primitive ova.

Coel
Gon.
Pr.o.
Mst.
4

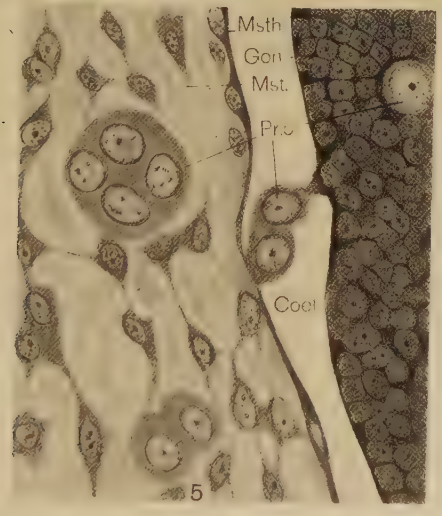
Msth
Gon
Mst.
Pr.o
Coel
5

PLATE 4

EXPLANATION OF FIGURE

6 Section through the right gonad of a one-hundred-and-twenty-hour chick which was castrated at a time prior to the establishment of the first intersomitic groove. The mesentery, the mesothelium and the gonad are devoid of sex cells. Note the peculiar foliage-like appearance of the gonad tissue. A few erythrocytes drawn to scale were inserted into the lower part of the figure. Bouin's fixation followed by iron-haematoxylin and eosin. The left gonad would have afforded a still better comparison with figure 4. The right one was chosen for the reason that the stroma-tissue was less dense than in the left. Both gonads are almost the same size. The mesothelium near the left gonad is very little thicker than that on the right. The left gonad contains no germ cells. × 800. *Coel.*, coelom; *Erth.*, erythrocytes; *Gon.*, gonad; *Mst.*, mesentery; *Msth.*, mesothelium of mesentery.

Coel.
Msth.
Mst.
Gon.
Erth.
6

THE RELATION OF AGE TO FERTILITY IN THE RAT

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THREE FIGURES

Data have recently been obtained that show the complete breeding history of a considerable number of female rats. An analysis of these data with reference to the question of fertility and its relation to age seems desirable, since literature dealing with litter size in rodents (bibliography in 'The Rat,' Donaldson, '15) gives very little information on this point and fails to record the entire litter production of even one pair of animals.

The breeding records of seventy-six females that produced a total of 585 litters are used in this study. The majority of the females (50) were piebald or 'hooded' rats; the rest were either 'extracted' albinos (15) or 'extracted' grays (11). All three strains were derived from the F_2 generation of a cross between the wild Norway rat (Mus norvegicus) and the domesticated albino (Mus norvegicus albinus). Mention is made of the kind of rats used merely as a matter of reference. The conclusions drawn from the results are doubtless applicable also to other strains of rats.

All of the females lived to be at least sixteen months of age, the oldest dying at the age of twenty-three months. Under the conditions existing in the animal colony of The Wistar Institute a rat is usually in its prime at the age of seven or eight months, and after reaching twelve months of age it is classed as 'old.' Very few individuals live for more than twenty months, although all animals are kept under environmental conditions that are seemingly well suited to their needs. The relatively early death of the animals is due, in part at least, to the fact

that seasonal changes in temperature in the region of Philadelphia render old animals very susceptible to pneumonia, the disease that invariably proves fatal to a rat of any age. In a more equable climate, like that of California, rats have been kept in good physical condition until they were four years old (Slonaker, '12).

In the rat the menopause usually appears at the age of fifteen to eighteen months (Donaldson, '15, p. 21). Data covering the litter production during the first sixteen months of life, therefore, may be assumed to show the actual fertility of the great majority of females. The word 'fertility' is here used as defined by Pearl and Surface ('09) to designate: "the total actual reproductive capacity of pairs of organisms, male and female, as expressed by their ability when mated together to produce (i.e., bring to birth) individual offspring." Fertility, according to this definition, is a much more comprehensive term than fecundity with which it is often confused. The latter, as suggested by Pearl and Surface should properly be used to signify only "the innate potential reproductive capacity of the individual organism as denoted by its ability to form and separate from the mature body germ cells."

Litter data for the three strains of rats are shown in table 1.

TABLE 1

Showing litter data for the three series of rats

	NUMBER OF BREEDING FEMALES	NUMBER OF LITTERS CAST	AVERAGE NUMBER OF YOUNG PER LITTER	TOTAL NUMBER OF YOUNG	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES
Piebald series	50	406	6.9	2798	1447	1351	107.1
Extracted albinos	15	88	6.2	548	279	269	103.7
Extracted grays	11	91	6.7	609	310	299	103.6
	76	585	6.7	3955	2036	1919	106.1

As table 1 shows, the corresponding records for the three series are very similar. The differences in regard to litter size and to the relative proportion of the sexes that are found are well within the limits of the variation that is always to be ex-

pected when the number of records is comparatively small. For further analysis, therefore, the data for the three strains have been combined. The entire series of records, arranged according to the age of the mothers when the litters were cast, is given in table 2.

The 'mean age of the females,' as given in the first column of table 2, is the median point of a thirty day period in the life of

TABLE 2

Showing the number of litters in the combined series, together with the sex ratios and the coefficients of variation for litter size. Data arranged according to the age of the females when the litters were cast

MEAN AGE OF FEM LES IN DAYS	NUMBER OF LITTERS CAST	AVERAGE NUMBER OF YOUNG PER LITTER	TOTAL NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALE	COEFFICIENTS OF VARIATIONS FOR LITTER SIZE
90	38	6.9	264	126	138	91.3	34.9
120	49	7.9	389	207	182	113.7	32.3
150	57	7.6	433	215	218	98.6	26.5
180	60	7.8	472	252	220	114.5	31.5
210	61	7.7	471	243	228	106.5	25.2
240	46	7.3	337	155	182	85.1	36.8
270	44	7.0	314	163	151	107.9	36.2
300	49	7.4	363	187	176	106.2	35.7
330	41	6.0	246	138	108	127.7	37.8
360	31	6.1	191	97	94	103.1	36.5
390	35	5.1	179	104	75	138.6	51.2
420	26	4.5	118	64	54	118.5	41.8
450	18	4.3	79	35	44	79.5	51.5
480	13	3.2	42	23	19	121.0	73.3
510	10	3.4	34	17	17	100.0	36.3
540	6	3.6	22	9	13	69.2	47.7
570	1	1.0	1	1			
	585	6.7	3,955	2,036	1,919	106.1	38.0

each animal, except in the two cases noted below. For example, the mean age '120 days' includes the records for all litters produced by females that were from 105 to 135 days of age when parturition occurred. The ninety day group is one exception to the above rule; it comprises litter records for a twenty day period only, as the youngest mother in the series was eighty-six days old when her first litter was cast. One female gave birth to a litter of one when she was 594 days old. For the

sake of uniformity this record is put under the mean age '570 days' which is thus extended to include a period of forty-four days.

The majority of female rats that are in good physical condition cast their first litters when they are about three months old. Thirty-eight of the seventy-six breeding females bore young before they were 105 days old; all of the remaining females,

Fig. 1 Graph showing, for the entire series, the relation of the age of the mother to litter production (data in table 2).

with four exceptions, threw litters before they reached the age of 135 days. As table 2 shows, the number of litters cast increased with the age of the mothers until the females attained the mean age of 210 days. After the age of maximum fertility was passed the number of litters cast decreased rapidly, and only a small proportion of the females bore young after they had reached the age of fifteen months.

The graph in figure 1, constructed from the litter data in table 2, shows the relation of the age of the mother to litter production.

The graph in figure 1 starts relatively high and rises rapidly to its maximum which comes at the 210 day period. The decline of the graph is much more gradual than its rise, and not until near the 360 day period does the graph drop to the level at which it starts. From this point the fall is more rapid, and the graph reaches zero after the females have attained the mean age of 570 days. Fecundity in the rat, measured solely by the number of litters cast by the females at different age periods, is thus found to accord remarkably well with the law formulated by Marshall ('10): "The fecundity of the average individual woman may be described, therefore, as forming a wave which, starting from sterility, rises somewhat rapidly to its highest point, and then gradually falls again to sterility." There can be no doubt that animals, in general, tend to follow a similar law, as the litter records for various species collected by Marshall, by Pearl ('13) and others have already shown.

Judging from the data in table 2 a female rat reaches the height of her reproductive capacity when she is about seven months of age. This age represents also the median point in the animal's breeding career. That is, one-half of the total number of her offspring are produced by the time she has reached this age and one-half are produced afterwards.

When the females have reached the age of eighteen months their reproductive activity is usually at an end, as the data in table 2 indicate. Donaldson ('06) has shown that the first year of a rat's life is approximately equal to thirty years of human life. On this assumption a female rat that is eighteen months of age corresponds physiologically to a woman of forty-five. The menopause evidently takes place in these two forms at about the same period in the life span of the individual, but there is no corresponding likeness as regards the age of puberty or of maximum fertility; both of these processes take place in the rat at a relatively much earlier period.

The third column of table 2 shows the average size of the litters cast by the females at different age periods. The litters of very young females contained an average of 6.9 young per litter. This is a smaller average number of young than is found

for any group of litters until the females have past the zenith of their reproductive activity. Such a result was to be expected, since a number of investigations, for instance those of Minot ('91) on guinea pigs and of Hammond ('14) on rabbits and pigs, have shown that the number of offspring produced by young animals breeding for the first time is usually below the number that is considered normal for the species, and also that litter size tends to increase for a time with the age of the female. The largest litters in the series were those produced by females with a mean age of 120 days. Litter size remained close to the maximum until the females were eight months old when a slight diminution in the number of offspring was noticed. A further decrease to an average of only six young per litter was found in the litters thrown by females that were one year old. Each succeeding month added to the female's life seemed to lessen the number of her offspring to a marked extent, and after the females were fifteen months old the mean size of the litters cast was only about three young per litter. Not infrequently the offspring of old females were born dead or soon died from neglect as the mothers seemed unable to suckle them.

There is, as yet, no standard for litter size in 'extracted' strains of rats with which the present series of records can be compared. Miller ('11) and Crampe ('84) give 10.5 as the average number of young in a litter of wild gray rats; but Lantz ('10), on examining a large series of animals, found an average of only 8.1 embryos in pregnant gray females. According to Crampe the average litter of albino rats contains 6.3 young; data for over 1000 litters, collected by King and Stotsenburg, give the mean number of young in albino litters as 7.0. According to the above observations litters of gray rats contain a greater average number of young than do those of albino rats. The 585 litters used in the present investigation contained an average of only 6.7 young. This seems to indicate that litter size in 'extracted' strains of rats is less than that in either of the pure strains from which the animals were derived. It must not be forgotten, however, that the litter size for the pure strains, as given above, was not obtained from the complete breeding rec-

ords of a number of females but from a random collection of litters cast by females of unknown age. Litter size in various strains of rats cannot be properly compared until litter records for the several strains have been collected in a similar manner.

The relation between the age of the mother and litter size is shown by the graph in figure 2. The data used in constructing this graph are given in table 2.

The graph reaches its maximum when the females are practically at the beginning of their reproductive activity (i.e., at

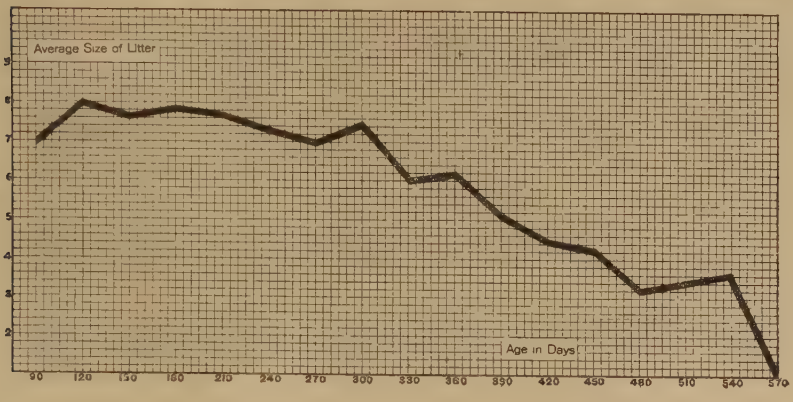

Fig. 2 Graph showing, for the entire series, the relation of the age of the mother to the average size of the litter (data in table 2).

120 days of age), and then declines very gradually approximating zero when the females are eighteen months old. Fertility in the rat, measured by the size of the litters cast, is thus found to be correlated with the age of the mother at the time that parturition occurs.

There is a possibility that the number of the pregnancy is a factor that influences the size of the litters cast. In order to analyze the data on this basis the records have been arranged according to the position of each litter in a litter series and are given in table 3.

When the data are arranged as in table 3 it is found that the second litter is the largest of the series. This result is in accord with the observations of Crampe ('84) and of King and Stotsen-

TABLE 3

Showing the number, the average size of the litters and the sex ratios when the data are arranged according to the position of each litter in a litter series

POSITION OF THE LITTER IN A LITTER SERIES	TOTAL NUMBER OF LITTERS	AVERAGE NUMBER OF YOUNG PER LITTER	TOTAL NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES
1	76	7.2	553	290	263	110.2
2	76	7.7	591	292	299	97.6
3	76	6.9	531	276	255	108.2
4	74	7.3	543	270	273	98.9
5	66	7.0	463	235	228	103.0
6	56	6.8	384	197	187	105.3
7	43	6.9	300	157	143	109.7
8	36	5.8	210	118	92	128.2
9	30	5.0	151	89	62	143.5
10	21	4.8	100	57	43	132.5
11	14	4.8	67	31	36	86.1
12	9	3.8	35	15	20	75.0
13	7	3.5	25	9	16	56.2
14	1	2.0	2	0	2	
	585	6.7	3,955	2,036	1,919	106.1

burg ('15) on the albino rat. The number of the pregnancy, up to five, does not seem to have a very marked effect on litter size. The first five groups of litters have an average of 7.2 young per litter, which is above the average size of litters of albino rats (7.0 young per litter) and considerably greater than the mean size of all the litters in the present series (6.7 young per litter). A slight decrease in size is noted in the sixth litter group, and in the succeeding litters the number of young diminishes steadily. Only exceptionally vigorous females are able to produce more than ten litters and these later litters rarely contain more than one to three young.

As a rule female rats begin breeding when they are three months old, and they will produce a litter each month for several succeeding months if they are in good physical condition. The second litter is cast, therefore, when the female is about four months old and the fifth litter is born when the mother is seven or eight months old. On referring to table 2 it is found that litters born when the females are four months old have a

greater average size than litters cast at any other age period, and that females reach the climax of their reproductive activity at about seven months of age. In both tables there is a rapid decrease in the size of the litters towards the end of the series. As far as the question of litter size is concerned the two tables are in complete agreement. Such a litter series as that in table 3 is necessarily an age series, and it is very probable that it is the age of the female and not the number of the pregnancy that is a determining factor for litter size.

The size of a newborn litter of rats depends, primarily, on the number of ova shed at a given period of ovulation that are capable of fertilization. Litter size, however, is not always indicative of the actual fecundity of the female, since the offspring born represent only that portion of the fertilized ova that were capable of normal development. Not infrequently the examination of a gravid female will show one or several fertilized ova in the uterus that are more or less atrophic and so incapable of developing into normal embryos (Huber, '15). Such ova are usually absorbed in situ, and only very rarely are monstrosities found among the normal newborn young. According to Hammond ('14), the lower fertility of young sows as compared with that of adult animals is due to the fact that not so many ova are shed at each period of ovulation. This explanation for the change in the fertility of swine is doubtless applicable also to a similar change in the fertility of rats and of other animals. Very probably the lessened fertility of old animals as compared with that of animals in their prime is due to the same cause. Whether abnormal ova are more frequent in old females than in young ones and so help to diminish the fertility in later life has not, as yet, been determined.

The last column of table 2 gives the coefficients of variation for the size of the litters cast by the females at different age periods. These coefficients show that size variation is considerably greater in the litters thrown by very young females than in the litters produced by females at the height of their reproductive activity when they are seven months of age. The latter

group of litters has the lowest coefficient (25.2) in the entire series.

As the number of litters cast after the females were a year old was relatively small, the coefficients for later litter groups can have little value. There seems, however, to be a very marked tendency for litters cast by older females to exhibit a greater range of variability in size than is shown by the litters of young females, the maximum variability appearing in the litters produced by females when they were about sixteen months old.

The entire series of litters gives 38.00 as the coefficient of variation for litter size. This coefficient is practically the same as that for litter size in the mouse, which is 37.5 according to the records collected by Weldon ('07), but it is 10 points less than the coefficient for the number of human offspring (Powys, '05). The coefficient of variation for fertility is very high in all mammals, apparently, being at least 25 per cent in the several cases where it has already been determined (Surface, '08).

Different females—even sisters from the same litter—show marked variations in the number and in the size of the litters they produce. Whether such differences depend upon the inheritance of various fertility factors, or whether they are due to environment or to individual peculiarities of the females themselves remains to be determined.

Table 4 shows the number of litters produced by the seventy-six females whose breeding records are used in the present study.

As shown in table 4, the range of variation in the number of litters produced by different females was from three to fourteen with an average of 7.7 litters per female. One of the two females that cast only three litters did not breed until she was six months old when she gave birth to a litter of seven. A second litter, with nine young, was born when the mother was eight months old, and a final litter, containing seven young, one month later. This female lived to be seventeen months old and she appeared to be in good physical condition until shortly before her death. The other female casting only three litters had a very similar breeding history. Some diseased

TABLE 4

Showing the litter production of 76 female rats

NUMBER OF BREEDING FEMALES	NUMBER OF LITTERS CAST
2	3
8	4
10	5
13	6
7	7
6	8
8	9
9	10
4	11
2	12
6	13
1	14
76	585

condition of the generative organs was doubtless responsible for the small number of litters produced by these two females, as investigations being carried on in the animal colony of The Wistar Institute by Dr. Stotsenburg show that sterility in a female rat is usually due to the formation of ovarian cysts or to degenerative changes in the uterus.

According to Crampe ('84), female albino rats, as a rule, do not produce more than four or five litters: records collected by Miller show that the wild gray rat has relatively more litters than the albino rat. The average of 7.7 litters per female, found in the present series of animals, is undoubtedly too high for the general run of females. Twenty-three of the seventy-six breeding females in this series had a total of five or six litters only, and it seems probable that this is about the average number of litters produced by female rats in general.

While six females had thirteen litters each, only one female gave birth to fourteen litters. This latter case is so unusual that it seems worthy of special note. The complete litter data are given in table 5.

This female, a piebald, gave birth to her first litter on February 7 when she was ninety-five days old. This litter was ex-

TABLE 5

Showing the litter production of a female piebald rat, that was born November 4, 1913, and died June 14, 1915

LITTER SERIES	DATE OF BIRTH	NUMBER YOUNG	MALES	FEMALES
1	February 7, 1914	11	5	6
2	March 11, 1914	13	5	8
3	April 3, 1914	8	6	2
4	April 30, 1914	9	5	4
5	May 23, 1914	9	6	3
6	June 20, 1914	10	2	8
7	July 14, 1914	11	5	6
8	August 12, 1914	6	2	4
9	September 10, 1914	10	6	4
10	October 15, 1914	10	9	1
11	November 23, 1914	4	3	1
12	January 28, 1915	3	2	1
13	March 26, 1915	3	3	0
14	April 28, 1915	2	0	2
		109	59	50

ceptionally large for the first litter of so young a female as it contained eleven young. The second litter, with thirteen young, was cast the following month. It is rather remarkable that both of these litters should be so much larger than normal, since, as a rule, a very large first litter is followed by a comparatively small one, unless at least two months intervene between the birth of the litters. The female cast two litters in April, and subsequently she gave birth to a litter each month until she was twelve months old. With one exception each of these litters was larger than the average litter of albino rats. A marked decline in fertility was noted after the female was a year old: the intervals between litters became longer and the size of the litters decreased. The fourteenth litter, which contained only two young, was cast when the female was about seventeen months old, and although the female lived to be nearly twenty-two months old she did not breed again. During this long period of reproductive activity a total of 109 young were born, 59 males and 50 females. The median point in this female's breeding career was the same as that for the entire group of

females, namely seven months, and she produced an average of 7.8 young in each litter.

An examination of the individual records for each of the remaining females in the series that gave birth to a very large number of litters i.e., from eleven to thirteen, shows that in every instance the first litter cast was large, containing from nine to eleven individuals. In those cases where females produced less than six litters the first litter cast, with one exception, never contained more than seven young. The number of records is so small that no definite conclusions can be drawn from them, but they seem to indicate that the size of the first litter cast is somewhat of an index of the fertility of that particular female: a large first litter indicating that the female, if she keeps in good physical condition, will produce more litters than the average run of females. Crampe states that the second of a rat's litters is always the 'best' and that this litter is indicative of the size of subsequent litters. This observation has been confirmed only in part by the present series of records: the second litter is the largest of the series, but the size of this litter is not as indicative of the later fertility of the female as is the size of the first litter cast.

Individual rats show as marked differences in the number of young produced at one birth as they do in regard to the total number of litters cast. Litters cast by some females are almost always relatively large. The female whose litter record is given in table 5, for example, cast but one litter in the first ten that contained less than seven young. Some females never have a litter that contains more than seven young, while others females cast a large and a small litter alternately.

The litter frequencies in the three series of rats are shown in table 6, the range in litter size being from one to sixteen.

In table 6, as in table 1, there are slight differences in the corresponding data for the three series of rats that may or may not prove to be significant when larger series of records are analyzed. Litters of eight young were most frequent in the piebalds and in the extracted grays, while six was the most common number of young in the litters of extracted albinos. The data for the

litter frequencies in the combined series is shown in the form of a frequency graph in figure 3.

The graph in figure 3 has two modes, one at the point of six and the other at the point of eight young per litter. The graph thus appears to be compound, and it is possible that one of the two modal points corresponds to the degree of fertility normal for the wild Norway rat and the other to the degree of fertility that characterizes the albino rat, since these are the two strains from which the animals used for this study were derived. As the material is probably heterozygous as regards the factors for litter size, it does not seem advisable to attempt any analysis of the curve. It is of interest in this connection to note that the graph for litter frequencies in swine, as given by Went-

TABLE 6

Showing litter frequencies in the three series

SIZE OF LITTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16
Piebalds	6	20	32	35	31	56	40	71	48	28	17	12	8	1	1
Extracted albinos		5	8	7	9	20	14	8	12	4	1				
Extracted grays		3	9	6	13	12	10	15	11	9	1	1	1		
	6	28	49	48	53	88	64	94	71	41	19	13	9	1	1

worth and Aubel ('16), has three modal points; one at four, a second at eight, and a third at twelve pigs per litter. The first mode corresponds to the degree of fertility in the wild hog, the third is close to that of the most fecund of the domestic breeds of swine, and the third probably represents a heterozygous condition.

Evidence regarding the relation of the age of the mother to the sex of her offspring is conflicting. Statistics collected by Bidder ('78) and by Punnett ('03) show that there is a great excess of boys among the children of very young mothers, the relative number of boys decreasing at subsequent births until the mother is thirty. Among children of old mothers (i.e., over forty) the sex ratio is again very high. In the horse Wilchens ('86) found a relation between the age of the dam and the sex of her offspring very similar to that existing, apparently,

in the human race. On the other hand, Schultze's ('03) investigations on mice indicate that the age of the mother has seemingly no influence whatever on the sex of her young.

According to the observations of King and Stotsenburg the normal sex ratio in the albino rat is about 107.5 males to 100 females. As there are no available data regarding the normal sex ratio in other strains of rats the sex ratio in the albino rat is here taken as the standard with which to compare the sex ratios found in the present series of animals.

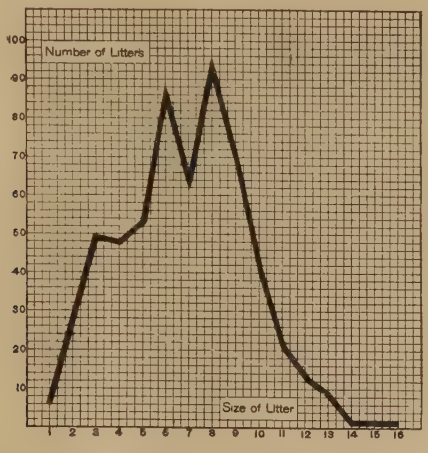

Fig. 3 Graph for the frequencies of litter size in the entire series (data in table 6).

Table 2 gives the sex ratios for the various litter groups when the data are arranged according to the mean age of the females at the time that the litters were cast. The sex ratios in litters belonging to closely related groups are so unlike that it would appear that there is no relation whatever between the age of the mother and the sex of her offspring. The sex ratio for the entire series of 3955 individuals is 106.1 males to 100 females. This shows that in the strains of rats used for this study the normal proportion of the sexes is about the same as that in the pure albino strain.

When the litter data are arranged according to the position of each litter in a litter series (table 3), the sex ratios obtained

for the individuals in successive groups of litters are not quite as diverse as those for related litter groups as shown in table 2. The sex ratio among the individuals belonging to the first litters of the series is higher than the standard, and in subsequent litter groups, up to the fifth, there is seemingly a tendency for the number of male offspring to decrease. A similar change in the sex ratios from the first to the fourth litter was noted by King and Stotsenburg in a series of litters cast by twenty-one albino females. Beginning with the fifth litter the sex ratios rise gradually until a maximum of 143.5 males to 100 females is reached at the ninth litter of the series. For the eleventh and subsequent litters, however, the sex ratios are much lower than the standard. From the sex ratios as given in table 2 it would appear that among the individuals of a litter series the sex ratio might be expected to start relatively high and then fall steadily until about the fifth litter, rise again gradually to a maximum at about the ninth or tenth litter and subsequently drop to a low level which is maintained until the female reaches the menopause.

The records under consideration are a special group selected solely because they cover the complete breeding history of a number of females that lived to an advanced age. Perhaps, therefore, they cannot be used legitimately to give evidence regarding the possible effects of the age of the mother on the sex of her offspring. From the data as given the only conclusion that can be drawn is that the age of the mother is not a dominant factor in determining the sex of her young. If, as Riddle ('16) maintains, sex is determined by the 'level of metabolism' in the fertilized egg, there is a possibility that the age of the mother may indirectly influence sex through its effects on the metabolic processes in the egg. Age has a profound influence on every tissue in the body, and its effects on the germ cells is a problem that must be attacked from a chemical standpoint, since it can never be solved by sex statistics however extensive they may be.

SUMMARY

1. Litter data covering the entire breeding history of seventy-six female rats are given in the present paper. All of the females belonged to 'extracted' strains that were derived from the F_2 generation of a cross between the wild Norway rat and the domesticated albino.

2. The material used comprises the data for 585 litters containing 3955 individuals, 2036 males and 1919 females. The average number of young in each litter was 6.7.

3. Fertility in the rat, measured by the total number of litters cast, increases with the age of the female up to the time that the animal is seven months old. There is a sharp decline in fertility after the female is a year old and, except in rare instances, the menopause has appeared by the time that the female is eighteen months of age.

4. Female rats reach the height of their reproductive activity when they are about seven months of age. This age also represents the median point in the animal's breeding career.

5. The age of the mother is a factor in determining the size of the litter cast. Litters of very young mothers are relatively small, and later litters are large until the female reaches seven months of age. Litter size diminishes with the reduction in the number of litters cast, and litters of very old females rarely contain more than three young.

6. The second litter is the largest of the series, the third and fourth litters are usually a little larger than the first.

7. The serial number of the pregnancy, up to the fifth, does not seem to alter the size of the litter to any great extent. The sixth litter cast, however, is smaller than the preceding ones, and the number of offspring decreases rapidly as the position of the litter in the litter series advances. It is very probable that it is the age of the mother, not the number of the pregnancy, that influences the size of the litters.

8. Coefficients of variation for litter size show that the litters cast by very young females have a greater range of variation in size than have the litters cast by females at the height of

their reproductive activity. From this point the range of variation in litter size appears to increase as the female grows older, and to reach its maximum in the litters cast when the females are sixteen months old.

9. For the entire series of litters the coefficient of variation for litter size is 38.00.

10. The total number of litters produced by different females varied from three to sixteen, with an average of 7.7 litters per female.

11. The majority of female rats probably produce from five to six litters only.

12. The size of the first litter cast seems to be somewhat of an index of the fertility of the female. If the first litter is very large the female will probably cast more litters than the average run of females, provided she remains in good physical condition.

13. The range in litter size was from one to sixteen. Eight was the most frequent number of young in the litters of the piebalds and of the extracted grays, while six was the most common number for the litters of the extracted albinos.

14. The sex ratio for the 3955 individuals in the series was 106.1 males to 100 females. This sex ratio is very close to the normal sex ratio for the pure albino strain (107.5 males to 100 females).

15. The sex ratios obtained for the various litter groups (tables 1 and 2) do not indicate that the age of the mother is a dominant factor in determining the sex of her offspring. Old females, however, seem to produce relatively more females than male young.

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TECHNIQUE NOTES

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I. THE APPLICATION OF BENDA'S NEUROGLIA STAIN

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This note embodies the results of some experiments with Benda's stain for neuroglia cells and fibers. The stain is, as is well known, the basic anilin dye, toluidin blue, used after a double mordantage of the sections in ferric alum and sodium sulphalizarinate. A previous mordantage of the tissues in a chrome solution, a 'chromation,' is an essential part of the method. Benda himself has employed two methods for producing this 'chromation.' The first (Benda, '00) was the use of Weigert's chrome alum bath followed by chromic acid, with tissue fixed in formalin. In the second method (Benda, '10), tissue fixed in alcohol was placed in 10 per cent nitric acid, then in 2 per cent potassium dichromate, and finally in 1 per cent chromic acid.

My results show that this 'chromation' may be obtained by using a chrome fixer, or better, by a dichromate bath after fixation; and the best results are produced by the use of a fixer containing a dichromate, followed by a dichromate mordantage. This is essentially the method used in preparing tissues for Weigert's copper hematoxylin, and it may be said that in general, sections which will take a good Weigert stain for myelin will also give good results with Benda's neuroglia stain. Tissues fixed in Zenker's fluid, in Zenker's followed by Müller's, in Helly's (Zenker-formol) followed by Müller's, in Müller's alone, in formalin followed by copper dichromate, and in the copper dichromate-sublimate-acetic mixture as used by Kingsbury, followed by copper dichromate, all have given good preparations with Benda's stain. It is worthy of note that the tissue fixed in Zenker's fluid alone gave the poorest neuroglia stain, and that good Weigert preparations were obtained from all the tissues, except this same one.

A number of sections of the spinal cord of an animal (a skunk happened to be available at the time) were fixed in copper dichromate-sublimate-acetic and mordanted in copper dichromate for periods from two to twenty-five days to determine the length of time required for the best 'chromation.' These all gave good results w th Benda's stain but the best preparations were obtained from tissues mordanted four to six days. Sections of this same material were also stained with Weigert's copper hematoxylin, and good preparations were obtained

from the tissue mordanted two days. After a longer stay in the copper dichromate solution, the myelin failed to stain with the copper hematoxylin, remaining yellow, while the neuroglia cells and fibers stained blue. With Benda's stain, the myelin after a short mordantage stains a light reddish brown, but after a longer mordantage remains yellow, unaffected by the stain.

From this it would seem that a short mordanting in dichromate solution is best for staining the myelin and a longer is best for neuroglia. After a prolonged mordantage the axis cylinders are brought out more clearly, apparently as a result of their 'chromation' for they did not stain with the copper hematoxylin. This does not quite agree with the results of Smith, Mair, and Thorpe ('08) who state that the order is myelin, axis cylinders, neuroglia.

This method of fixation—Helly's fluid (Zenker-formol) followed by Müller's or copper dichromate-sublimate-acetic followed by copper dichromate—has given excellent results with tissues from a number of animals: cat, dog, man, mouse, rat, skunk. The spinal cord was used in each case, and in addition, in one form (dog) the optic nerve was taken.

These results would show that for the 'chromation' four to six days in copper dichromate (2.5 per cent solution) is equivalent to two to four weeks in Müller's fluid or plain potassium dichromate solution. There is apparently little choice between them; possibly the tissues mordanted in copper dichromate are a trifle less brittle. A 2 per cent solution of potassium dichromate works as well as Müller's fluid.

My results with material fixed in this way (Müller's after Helly's or copper dichromate after the copper dichromate-sublimate-acetic mixture) have been better than with tissues treated as Benda recommends. The preparations correspond to his description; neuroglia fibers and nuclei of neuroglia cells, deep blue; cytoplasm of neuroglia cells, paler blue or purplish; myelin, reddish brown; axis cylinders darker red; connective tissues light red or pink; nerve cells reddish or purplish; and Nissl bodies darker purple.

Benda's method of staining was used, and is given for reference. Paraffin sections 5 to 8 μ are treated as follows:

1. 4 per cent ferric alum, twenty-four hours.
2. Running water, ten–twenty minutes, followed by several changes of distilled water.
3. Amber-yellow solution of sodium sulphalizarinate, twenty-four hours (saturated solution of sodium sulphalizarinate (Kahlbaum) in 70 per cent alcohol, 1 cc., distilled water, 100 cc.).
4. Several changes of distilled water, which is then absorbed with tissue paper.
5. 0.1 per cent aqueous solution of toluidin blue, heated (on the slide) until it steams, and allowed to cool, fifteen minutes or more.
6. After rinsing in distilled water the sections are treated for a few seconds with acidulated alcohol (70 per cent alcohol, 100 cc., concentrated hydrochloric acid, 6 drops). The length of time required varies

with the different methods of mordanting, and is best determined by trial.

7. After the acid alcohol is absorbed with tissue paper, the sections are rapidly dehydrated with absolute alcohol.

8. The sections are then differentiated with creosote under control of the microscope. This usually takes several minutes. If more than ten seems necessary, it may be well to remove the creosote with absolute alcohol and treat with acid alcohol again for a few seconds; then the sections may be dehydrated and differentiated with creosote as before.

9. The creosote is absorbed with tissue paper and after several changes of toluene or xylene, mounted in balsam or damar. To prevent the fading of the stain, it is necessary to remove the creosote pretty thoroughly.

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II. SOME USES OF MALLORY'S CONNECTIVE TISSUE STAIN

H. M. KINGERY

Mallory's anilin blue connective tissue stain is of course well known for the purpose for which it was intended, but it has been found quite useful for other purposes as well. As usually employed for collagen fibers, Zenker material is used. I have found that this stain used with tissue fixed in picro-aceto-formal (Bouin's fluid) gives a very pretty differentiation for skeletal muscle, distinguishing clearly the isotropic and anisotropic bands.

In practice, a thin muscle is moderately stretched and pinned out flat on a cork which is then floated upside down on the fixer. The picric acid is sufficiently removed after washing about a week in alcohol. Thin paraffin sections (4–6μ) are stained according to Mallory's directions (five minutes in the acid fuchsin solution and then twenty minutes in the anilin blue solution; differentiation in 95 per cent alcohol, dehydration in absolute alcohol, toluene or xylene, balsam or damar). Differentiation proceeds rather slowly and may be watched under the microscope. In a finished preparation the dark band (anisotropic) is stained blue and the light band (isotropic) is light red or pink. Hensen's disc (M) appears light in the middle of the blue band and Krause's membrane (Z) is deep red in the middle of the pink band.

This method of fixation and staining when applied to insect materia differentiates very nicely chitinised from non-chitinised cuticula. The chitinised is stained red and the non-chitinised is a clear blue. This method was applied particularly to the intestine of the grasshopper and besides differentiating the cuticula it brought out the striations of the muscle fibers of the muscular coats with almost diagrammatic clearness.

With this same fixation (picro-aceto-formol) Mallory's stain also brings out the connective tissue very clearly. And I have found that the stain may be used with good results after a number of fixers—alcohol, Carnoy's 6–3–1, formalin—if the sections are placed for a short time in picro-aceto-formol and then washed, before staining. Aqueous and alcoholic solutions of picric acid also give fairly good results as 'mordants,' but not so good as picro-aceto-formol. The results obtained by this method compare very favorably with those secured after Zenker fixation.

The formulae follow.

Picro-aceto-formol

Saturated aqueous solution of picric acid	75 parts
Formalin	25 parts
Glacial acetic acid	4 parts

Mallory's anilin blue stain

Solution A.	
Acid fuchsin	0.2 gram
Distilled water	100.0 cc.
Solution B.	
Grübler's water soluble anilin blue	0.5 gram
Orange G	2.0 gram
1 per cent aqueous solution of phosphomolybdic acid	100.0 cc.

III. THE USE OF THE VAN WIJHE METHOD FOR THE STAINING OF THE CARTILAGINOUS SKELETON

GUSTAVE J. NOBACK

While the Schultze and the Spalteholtz clearing methods for bone are well known and frequently used, it would seem to the writer that the van Wijhe staining and clearing method for cartilage, which nicely complements the Spalteholtz method in the study and demonstration of the development of the skeleton, is not so well known and appreciated. I venture therefore to call attention briefly to its value, having used it during the past three years with satisfactory results.

The method is very simple; embryos or other material to which it is to be applied should be preserved in alcohol or (better) formalin. The specimen is next placed in 67 per cent alcohol with 1 per cent of hydrochloric acid added, for several days or a week. It is then transferred to the same solution plus 0.25 per cent of methylene blue in which stain it remains for a week or two weeks, until thoroughly stained. Toluidin blue may be used instead of methylene blue if preferred (Lundvall

'04,'12). It is then retransferred to the acid alcohol, which is changed at intervals of one or two days or when markedly colored. In this the specimen remains until the color is nearly entirely removed from all parts save the cartilage which remains deep blue. To remove the acid it should be washed for several days with changes of 82 per cent (85 per cent) alcohol and then dehydrated by passing up through 95 per cent alcohol, absolute alcohol, equal parts of absolute alcohol and benzene, into benzene, changed at least once, in which it may remain, or it may be mounted in xylene damar or Canada balsam.

The method is particularly serviceable in demonstrating the development of (a) the sternum and ribs, (b) the auditory ossicles, Meckel's cartilage and Reichert's cartilage, (c) the chondrocranium, (d) the cartilage in the developing bones of the extremities, etc.

As has been indicated at the beginning, the method supplements satisfactorily the Spalteholtz method in which the bone has been stained red in the usual way by means of alizarin. Two embryos, or the two halves of the same embryo carefully cut as nearly as possible in the median plane, may be run through, for bone (according to the Spalteholtz alizarin method) and for cartilage respectively, the skin, central nervous system and viscera having been removed to clarify the view. One arm or leg may be stained for bone and the other for cartilage, etc.

A solid mounting medium offers so many advantages over a liquid one that where possible it was used, damar balsam in xylene solution being preferred to Canada balsam because of its lighter color. By using glass supports for the cover-glass, such as small pieces of glass rod, etc., and using care in adding successive amounts of xylene damar, solid mounts of quite large specimens may be made on glass slides or plates. In this way specimens such as arms of the same embryo stained for bone and cartilage respectively may be mounted side by side or in parallel series to show advancing stages of development. Glycerin-jelly may be used as a solid mounting medium in the Schultze method, or with alizarin stained bone, but it may not be used with specimens stained for cartilage with methylene or toluidin blue. In the use of liquid mounting media, it was found that the benzyl benzoate and oil of wintergreen mixtures used in the Spalteholtz method were not so useful for mounting specimens stained for cartilage by the van Wijhe method, since fading was apt to result. Benzene was found to be the most serviceable mounting medium. Lundvall has used for larger specimens benzene four parts and carbon disulphide one part (see also Lundvall, 1912).

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IV. A CONVENIENT METHOD OF ORIENTATION IN PARAFFIN IMBEDDING WHEN PAPER TRAYS OR BOXES ARE USED

B. F. KINGSBURY

The following simple method has been used by the writer occasionally for the past fifteen years or so and since I do not recall ever having seen it mentioned, a brief description may not be out of place. The paper box method of paraffin imbedding which I use quite generally is of course well known and described in most books of technique (cf. Lee, Vade-mecum, p. 77).

The procedure in obtaining the orientation is the following. By means of a moderately soft lead pencil direction lines or, if desired, the outline of the specimen or embryo (traced from a x 1 photograph), are marked on the inside of the bottom of the box chosen or usually on the paper before it is folded. The box is then floated on cold water and the melted imbedding paraffin poured in. The specimen is then at once transferred from the melted infiltration paraffin to the box. By the time this has been done a thin translucent layer of solidified paraffin covers the bottom of the box, the orientation lines upon the paper showing through. The specimen is arranged as desired according to the orientation lines or upon the outline and the paraffin allowed to cool, etc. Subsequently after removing the paper from the cold block it will be found that the pencil mark is on the paraffin block which then may be trimmed and by it easily oriented for sectioning.

ON THE ELECTIVE STAINING OF THE ERYTHROCYTE

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Today we are in possession of numerous excellent staining methods applicable to microscopic researches of the blood. The anilin dyes have been especially serviceable in the coloration of the red blood cells, and among these Eosin and Orange G deserve special mention. Solutions combining a number of dyes, for instance the triacid mixture (Ehrlich-Biondi), eosin methylblue (May-Grünwald), methylazure methylblue eosin (Giemsa), have rendered excellent service in the hands of many investigators.

However these dyes are designed more particularly for the staining of film specimens of blood, since in staining sections they color not only the erythrocytes, but also the plasmic substances; in a strict sense they are, therefore, not the elective stains for erythrocytes. The need of an elective stain for the hemoglobin bearing erythrocytes is often felt in researches dealing with the genesis of blood cells and in many other types of the histological investigations.

I have recently discovered a method of the elective tinction of the erythrocyte. The finding is based on the fact that the phosphomolybdic acid lac of alizarin stains among several tissue elements only haemoglobin. After mordanting with phosphomolybdic acid the great majority of the tissues of the animal body lose the property of staining with molybdenum alizarin lac while the erythrocyte or more particularly haemoglobin is colored with it. In this regard the method here recorded may be regarded as a new method for the microscopic determination of haemoglobin.

The various steps of the method are as follows:

The material may be fixed in formalin, sublimate, potassium bichromate, etc.

1. The sections are transferred to distilled water.
2. Mordant in 10 per cent phosphomolybdic acid solution for 30 seconds to 2 minutes.
3. Wash in water.
4. Stain in following mixture for 20 minutes to 20 hours. Sodium sulfalizarinate, saturated aqueous solution—100 cc. 10 per cent phosphomolybdic acid, aqueous solution—30 cc. (10–50 cc.).
5. Wash in water.
6. Alcohol.
7. Xylol, balsam.

It is not necessary to prepare the staining solution a short time before using. A solution kept for one-half year, exposed to daylight, gave excellent results. On mixing the phosphomolybdic acid and sodium sulfalizarinate solutions the yellowish brown color first observed later changes to one of bright orange red.

Attention is called to the fact that on staining sections according to this method, the erythrocytes of vertebrates, the nuclei of the erythrocytes excepted, are durably stained a light to dark orange red, other tissues remaining unstained. The method is thus differential. Some-

times the nuclei and protoplasmic substances, especially in materials fixed in bichromate of potassium, take the stain a little, but it is easy to distinguish the bright orange red color of erythrocyte from the dirty yellowish brown color of nuclear chromatin or from other protoplasmic substances, for in the latter the color bleaches gradually. The connective tissue fibrils and osseous tissue are colored a bluish tinge, increased in intensity by longer staining so that excellent double staining, with brilliant contrast in orange red and blue may be obtained.

It remains to be considered whether the molybdenum alizarin lac stains haemoglobin or some other structure of the erythrocyte. To determine this question the following experiment was undertaken.

On a spot on two slides the chemical pure hemoglobin (Merk, Darmstadt), was spread and near it a section of liver fixed in formalin. Both were allowed to dry. One slide was now mordanted in the phosphomolybdic acid solution, the other not. Both were then stained in the alizarin molybdenum lac. On the slide mordanted we observe that the liver cells were not stained while the haemoglobin was colored a very dark orange red. On the unmordanted slide the liver cells were colored brilliant red and the haemoglobin a deep orange red. This observation may serve to show that the erythrocyte or haemoglobin represent the substance which stains after mordanting of phosphomolybdic acid by molybdenum alizarin lac.

From the facts given it would seem that molybdenum possesses the property of effecting animal tissues so that mordanting by it diminishes or entirely deprives them of their staining property, this with the exception of haemoglobin. It is a question whether the molybdenum alters the majority of tissues, haemoglobin excepted, or whether there exists a peculiar affinity between the haemoglobin and this lac. It may be of interest to determine precisely the chemical relations of both.

It is recommended that before or after the staining by the solution given the sections be treated with some nuclear dye. Haematoxylin may be used for this purpose, the section being first stained in this dye. On the subsequent use of haematoxylin, staining of connective tissue fibrils is obtained by reason of the formation of an haematoxylin molybdenum lac as in the Mallory's stain.

In conclusion it may be stated that as with the solutions so with the stained preparations, they are durable. The slides made one year ago and kept in the half dark are as yet unbleached.

After the present work was completed and ready for publication, attempts to make alcoholic solutions were undertaken. The alcoholic saturated solution of the sodium sulfalizarinate changes a little its color on the addition of the phosphomolybdic acid. The procedure is the same as described for aqueous solutions. By this modification the length of time required for staining has been considerably shortened and the coloration seems more certain. On the durability of both solutions and preparations, when alcoholic solutions are used, a future communication will give information.

The alcoholic stain has been prepared by mixing the two following solutions: Sodium sulfalizarinate saturated alcoholic solution, 100 cc.; 10 per cent phosphomolybdic acid aqueous solution, 1 to 2 cc.

NEUTRAL RED AS A CELL STAIN FOR THE CENTRAL NERVOUS SYSTEM

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The method described below has been used for eight years for the study of the tigroid content of cells as well as the size, form and grouping of cell bodies. As a 'Nissl method' it gives better results with formalin material than methylene blue or toluidin blue. The method is simple and the stain is permanent and is suitable for photography.

The stain is made up in a 1 per cent aqueous solution and kept for months or years until thoroughly ripened. The ripening process is hastened by exposure to the air or by boiling but I know of no way to obtain a satisfactory staining solution in a few days. A good stain can be had after a few months; a better one after three or four years. A 1 per cent solution is diluted as required and the dilute stain may be used over and over again.

The stain is used with either celloidin or paraffin sections. Differentiation is carried out in alcohol. The lower grades of alcohol differentiate slowly enough to allow one to examine the sections under the microscope. The higher grades wash out the stain and the sections should remain in these grades only as long as necessary to secure dehydration. If differentiation has been completed in 70 per cent alcohol, it may be carried too far while the sections are being dehydrated in the higher grades.

The clearing of the sections requires care, as any alcohol left in the sections will remove the stain. Also, some of the common clearing agents injure the stain while others soften celloidin. For paraffin sections xylol is safe and satisfactory. For celloidin sections castor oil and bergamot oil are the only common clearing agents which have proved constantly reliable. Carbol-xylol ruins the stain at once and cedar oil, clove oil, cajepat oil, oil of thyme, and anilin oil have all been unsatisfactory for one reason or another, at least in certain samples. After clearing in bergamot or castor oil it is well to rinse the sections in xylol. The mounts harden more rapidly and seem a little more brilliant and transparent. If castor oil is used the sections can be handled much more easily if the oil is thinned by addition of one part of xylol to two or three of castor oil. The xylol also counteracts any tendency of the castor oil to soften the celloidin. It is most convenient in handling large sections to float them on glass slides in the last grade of alcohol and pass them through the clearing agent on the

slide. In all these particulars the method is more difficult with large and, especially, thick sections. Sections 50 microns in thickness through the whole brain of a newborn babe have been perfectly stained by this method, while sections 100 microns thick through the entire adult human brain could not be differentiated and cleared uniformly.

The steps in the process may be stated as follows:

Material fixed in formalin or alcohol. Old formalin material gives good results.
Paraffin or celloidin sections.
Aqueous neutral red Grübler (well ripened) diluted to one-fourth or one-tenth of 1 per cent.
Use warm 10 to 30 minutes, or cold 12 to 24 hours. Differentiate in 50 or 70 per cent alcohol. Dehydrate rapidly in higher grades.
Clear paraffin sections in xylol; celloidin sections in castor-xylol or bergamot oil.
Mount in dammar or balsam. Clearing and mounting media must be neutral.

Neutral red does not work well after chromic salts and hence can not be used as a secondary stain after Weigert. The stain does work admirably, however, after either the Cajal or Bielschowsky process on formalin or alcohol material. Some beautiful preparations have been obtained by treating with neutral red as above sections in which the silver stain has attacked chiefly the fibers. This enables one to study the relations of cell masses to fiber bundles and to see the origin of a silver-coated axone from the cell-body stained by neutral red.